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VOLUME I
SUMMARY STATEMENTS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Annual Report of the Biological Psychiatry Branch

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Robert M. Post, M.D., Chief

The Biological Psychiatry Branch consists of several clinical and basic science research endeavors. The clinical efforts are centered on the inpatient facility of the 3-West Clinical Research Unit and in a variety of outpatient settings. Dr. T.W. Uhde directs studies of panic anxiety and affective disorders on the inpatient unit and conducts an extensive series of clinical, biological, physiological, and pharmacological investigations in a large cohort of outpatients with anxiety disorders studied in the Ambulatory Care Research Facility (ACRF). In addition to his role as Clinical Director and head of the Consultation and Liaison Service, Dr. D.R. Rubinow studies endocrine and peptide mechanisms involved in the affective disorders and has a large outpatient clinic for the study of menstrually-related mood disorders. A major Section within the Branch, that of Clinical Neuroendocrinology, headed by Dr. P.W. Gold, has separated from the BPB so that Dr. Gold can head up his own independent branch with inpatient studies focused on 3-East. The Branch Chief heads up a clinical research program (Section on Psychobiology) focused on acute and long-term treatment of recurrent manic-depressive disorder, and in particular on treatment alternatives to lithium carbonate. Current work involves studies of the psychotropic effects of anticonvulsant drugs and their possible mechanisms of action.

Basic science components of the Branch are integrally related to the clinical research themes. Dr. Uhde conducts laboratory studies of neurotransmitter and receptor mechanisms in clinical and preclinical materials. He is studying an animal model of panic anxiety in genetically anxious pointer dogs. Dr. Rubinow's laboratory efforts derive from his projects in consultation-liaison regarding mechanisms of interleukin-2-stimulated endocrine secretion and the mechanisms of its sensitization, as well as studies of brain somatostatin levels and receptor function. Dr. S.R.B. Weiss heads up studies on kindling and behavioral sensitization in the Section on Psychobiology. Animal models of psychomotor stimulant-induced behavioral sensitization and electrophysiological kindling are studied, as they may provide insights into the mechanisms underlying evolution of pathological behavior in response to the same stimulus input over time. In addition, Dr. A. Pert heads the Unit on Behavioral Pharmacology focused on understanding mechanisms of drugs of abuse, and in particular on neural substrates underlying reward behavior and other phenomena directly pertinent to psychiatric conditions where alterations in hedonic behavior are characteristic.

Dr. P. Marangos headed the Unit on Neurochemistry, focused on understanding basic receptor and subreceptor mechanisms involved in psychotropic drug actions, such as mechanisms of action of carbamazepine, and receptor effects of adenosine, benzodiazepines, and calcium channel-active agents. Work in this Unit also focused on understanding the basic mechanisms involved in behavioral sen-

sitization and kindling as far as they represent models for learning and memory. Dr. Marangos has recently left the NIMH to head up a CNS division of Gensia Pharmaceuticals in San Diego.

Unit on Anxiety and Affective Disorders (Thomas W. Uhde, M.D., Chief)

Dr. Uhde and his co-workers have developed novel life-charting techniques to study the longitudinal course of panic disorder. His systematic studies demonstrate that the typical onset of the illness occurs first with panic attacks, generally in adolescence or early adulthood, followed by the gradual emergence of additional symptoms, including anticipatory anxiety and agoraphobia, as well as brief periods of major depression in approximately 50% of patients. At the onset of panic disorder, patients experience more life events than age- and sex-matched controls. Patients who experienced a major separation or loss (i.e., death of a spouse) were at greater risk for development of a subsequent major depression compared with panic disorder patients who had not experienced such a loss. In contrast, other types of stresses did not influence the nature, course, or severity of panic-anxious behaviors. Another line of investigation has suggested that the presence of social anxiety (social phobia) may be a risk factor for the development of major depression in panic disorder, while the presence or absence of agoraphobia may be unrelated to the development of depression.

An unexpectedly high lifetime prevalence of obsessive-compulsive symptoms was found in 27% of panic disorder patients. Patients with additional obsessive-compulsive symptoms compared with those without had a higher rate of depression, alcoholism, and drug abuse, and were less responsive to traditional antipanic medications. Moreover, a similar pattern was observed in the first-degree relatives of these patients with obsessive-compulsive and panic-anxious symptoms combined; i.e., there was a greater prevalence of depression, drug abuse, and alcoholism in the first-degree relatives.

These findings are of particular interest in that they do not support the popular notion that depression is simply a secondary complication (demoralization phenomenon) of agoraphobia rather than a co-morbid condition connected to panic disorder.

A series of investigations have focused on the circadian timing of anxiety and panic symptoms. These studies also challenge another long held notion that panic disorder and anxiety neuroses worsen over the course of the day. In fact, in a prospective study, it was found that symptoms of generalized anxiety, phobic anxiety, and even the frequency of panic attacks occur more in the first eight hours of the awake day compared with the last eight hours prior to sleep. Diurnal variations in these symptoms are more profound in patients with a past history of depression compared with patients without such a history.

Other interesting phenomenological characteristics of panic anxiety disorders have been elucidated. In particular, there is a high incidence of psychosensory phenomena, typically experienced by patients with complex partial seizures (psychomotor epilepsy). These findings suggest that limbic

and temporal lobe substrates may play a role in the neurophysiology of panic disorder. However, Dr. Uhde and his group have documented that patients with panic disorder do not have abnormal EEG patterns, even when studies are conducted with nasopharyngeal leads and following sleep deprivation. It is also of interest that psychosensory symptoms do not predict clinical response to the anticonvulsant carbamazepine.

A major thrust of investigations are the comparative phenomenology, neurobiology, and pharmacoresponsivity of patients with panic disorder and affective illness, particularly in light of the common response of both groups to the tricyclic and monoamine oxidase inhibitor antidepressant drugs. Dr. Uhde has found common endocrine and biochemical abnormalities, including blunted ACTH response to CRF, blunted TSH response to TRH, paradoxical growth hormone response to TRH, and blunted growth hormone, cortisol, and blood pressure responses to clonidine in both panic disorder and affective disorder patients. In contrast to abnormalities in patients with affective illness, Dr. Uhde and his group have found normal degrees of urinary excretion of free cortisol, dexamethasone suppression, and measures of platelet [^{3}H]imipramine binding in panic disorder. He has, in addition, found a differential clinical response to one night's sleep deprivation. This procedure is associated with improvement in some 60% of depressed patients, but 58% of panic disorder patients actually worsen during the morning following total sleep deprivation.

Profiles of EEG-monitored sleep also demonstrate differences between these two patient populations. Those with panic disorder have only mild decreases or normal REM latencies; they have normal REM densities and REM percentages. Taken together, the clinical, biochemical, and endocrinological data suggest that panic and depressive disorders share a number of common biological and pharmacological markers, but that the two conditions can be clearly differentiated by selective probes such as response to dexamethasone, normal degrees of urinary free cortisol secretion, sleep deprivation responsivity, and sleep polysomnography.

Findings of both clinical and theoretical significance have been elucidated in the area of sleep-related panic. Drs. Uhde and Mellman have found that sleep-related panic attacks occur during stage II or early stage III (slow wave) sleep and not during rapid eye movement (REM) sleep, which is associated with dreaming. These first reports of systematic studies of sleep-related panic, soon to be published in the Archives of General Psychiatry, do much to place the phenomena of panic attacks on a physiological basis and clearly indicate that panic attacks can arise out of nonREM sleep and be dissociated from the symbolism, cognitions, and imagery of daytime awakening or even of dreaming. While they do not rule out the possibility that interoceptive conditioning may play a role in the maintenance of sleep-related panic attacks, the findings that a group of panic-disorder patients experience sleep-related panic attacks at the outset of their illness, suggest that this is an unlikely possibility. In addition to the systematic polysomnographic studies of sleep related panic, Drs. Uhde and Mellman have found, on the basis of questionnaire studies, that rather than being extremely rare, sleep-related panic attacks occur with a lifetime prevalence of 65% in patients with panic disorder. Four percent of panic disorder patients have as frequent or more frequent sleep-

related panic attacks than daytime panic. Those panicking during sleep were significantly more likely to have problems with insomnia and depression and to be vulnerable to relaxation- and sleep deprivation-induced panic attacks. Patients with prominent sleep-related panic develop secondary insomnia (i.e., fear of sleep) in a fashion that appears very similar to the development of agoraphobia in patients with daytime panic attacks.

In addition to the altered pharmacoresponsivity of patients with panic disorder compared with controls (see caffeine data below), the current studies of sleep-related panic do much to place panic disorder in the realm of an autonomous physiological dysfunction (distinct from, but akin to, epilepsy), and, in conjunction with recent studies showing an extraordinarily high incidence and prevalence of panic disorder in the general population, should do much to destigmatize this disabling psychiatric illness and propel our further studies of its fundamental mechanisms.

Dr. Uhde and his group have pioneered in studies of novel pharmacological interventions in panic disorder. 1) He has found that the alpha-2 agonist clonidine exerts acute and clinically robust anxiolytic effects which are generally not maintained during chronic administration. Dr. Uhde has reported the first clinical trial of the calcium channel blocker verapamil in the treatment of panic disorder patients. Significant antianxiety effects were observed during this double-blind clinical trial in 14 patients. Eighty-two percent of patients had a decrease in the number of panic attacks during the last four weeks of verapamil treatment, compared with four weeks on placebo preceding verapamil. These promising findings provide preliminary evidence for the anxiolytic and antipanic effects of the calcium channel blockers which should lead to further clinical studies and elucidation of precise mechanisms responsible for these clinical effects. 2) The first double-blind, placebo-controlled trial of the anticonvulsant carbamazepine has also been conducted by Dr. Uhde's group. Fourteen patients with panic disorder were studied and although mild to moderate improvement was observed in 65% of panic disorder patients, there was no overall statistically significant effect on measures of Zung anxiety or generalized anxiety measured on the SCL-90 scale. These data suggest that carbamazepine, which exerts its anticonvulsant effects through the peripheral-type benzodiazepine receptor, in contrast to drugs such as clonazepam, diazepam, and alprazolam, which exert their effects through the central-type benzodiazepine receptor, may not be an efficacious drug in the treatment of panic disorder patients. Clearly, these data are of considerable mechanistic interest, even though it does not appear that carbamazepine will be a clinically useful treatment agent in the majority of patients with typical panic anxiety syndromes. In this regard, it is of interest that carbamazepine exerts adenosine antagonist properties at the adenosine receptor, similar to caffeine. It is possible that such a mechanism could be related to carbamazepine's lack of efficacy in panic disorder, in light of the panicogenic properties of caffeine and related adenosine antagonists.

Dr. Uhde is widely recognized for his studies of caffeine in patients with panic disorder, affective illness, and normal controls. He has demonstrated that 40% of panic disorder patients experienced a panic attack following oral administration of caffeine (480 mg) while no normal volunteers

experienced high levels of anxiety or panic at this dose. However, at higher doses, two of 12 normal volunteers developed unequivocal panic attacks following the administration of 720 mg of caffeine. These data strongly suggest that high doses of caffeine can be panicogenic and that panic patients have differential responsivity, if not sensitivity, to caffeine compared with normal volunteer controls. These data provide a direct confirmation of an earlier questionnaire self-report study that suggested that panic patients had difficulty tolerating caffeine-related products such as coffee and had given up drinking these agents because of their psychoactive properties at a much higher rate than patients with affective illness or normal volunteer controls. In related studies, Dr. Uhde has found that caffeine produced dose-related increases in cortisol, glucose, and lactate in normal volunteers. Plasma cortisol lactate levels were significantly higher in the patients compared with the normal controls. The triazolobenzodiazepine drug, alprazolam, which has demonstrated antipanic effects, has been shown to block caffeine-induced panic and to block the rise in cortisol and lactate in panic disorder patients.

Because of these provocative findings with caffeine, a comparative study of the anxiogenic effects of caffeine and metachlorphenylpiperazine (mCPP), a serotonin agonist that had previously been reported to produce anxiety in panic disorder patients and normal volunteer controls, was investigated. This study represents the first attempt to separate anxiogenic probes with different mechanisms of action in the same patients. Preliminary data from this study indicate that both caffeine (480 mg) and mCPP (0.5 mg/kg) had anxiogenic effects, although caffeine produced nonsignificantly greater increases in anxiety on all rating scales. Both agents produced comparable increases in plasma cortisol but only mCPP produced increases in plasma prolactin. Taken together with previous findings of altered alpha-2 noradrenergic responsivity to a challenge with yohimbine, these findings suggest a role for noradrenergic, serotonergic, and adenosinergic/benzodiazepine receptor systems in the neurobiology of panic disorder.

Based on the findings of altered responsivity to the adenosine antagonist caffeine in patients with panic disorder, the first clinical trial of an adenosine uptake inhibitor, dipyridamol, has been initiated. Preliminary results suggest that some patients are experiencing antipanic and antianxiety effects of this agent, which enhances adenosine function. Two other studies also support an important role for caffeine and, possibly, adenosinergic mechanisms in endocrine regulation and anxiety expression. Dr. Uhde had previously demonstrated that 480 mg of caffeine given at 2 PM on the day following a dexamethasone suppression test induced abnormal escape from dexamethasone suppression in 20% of patients previously demonstrated to be normal suppressors. These data suggest the possibility that caffeine and other dietary constituents may play an important role in this widely used clinical test in psychiatry.

Studies of the effects of repeated daily caffeine administration have been conducted in patients with panic disorder and in normal controls. Preliminary analysis of the data suggests that tolerance occurs to repeated caffeine administration in the normal volunteers, but that a different pattern occurs in patients with panic disorder. Not only do they demonstrate a delayed

tolerance pattern, but after one or more days of caffeine administration, there appears to be an increase in anticipatory anxiety. These data provide one of the first systematic studies of the longitudinal adaptation to repeated pharmacological challenges, which may be important in elucidating alterations in adaptive responses, in addition to previously demonstrated alterations in acute responsivity to caffeine.

New findings have been observed with challenges of panic anxiety patients with yohimbine, an alpha-2 adrenergic antagonist that increases firing of the locus coeruleus. Baseline arousal and anxiety have been shown to predict vulnerability to yohimbine. Moreover, panic disorder patients, but not normal controls, can adequately and accurately discriminate between yohimbine and placebo, suggesting that alterations in noradrenergic function may be more finely discriminated and serve as an important interoceptive cue during naturally occurring panic attacks. From a biochemical perspective, yohimbine produces significantly greater increases in cortisol but not norepinephrine, MHPG, blood pressure, or heart rate in panic disorder patients compared with normal controls. In relationship to the correlates of noradrenergic function, it is interesting that the yohimbine tends to have a main drug effect on MHPG but not norepinephrine. This suggests a potentially interesting dissociation between norepinephrine and MHPG as markers of noradrenergic function, which could help to explain various discrepant findings in the literature and also propel studies of the mechanisms underlying this paradoxical uncoupling.

Dr. Uhde and his group have also systematically studied the role of dopaminergic function in panic and anxiety disorders. He has elucidated possible changes in the dopamine metabolite homovanillic acid (HVA) in relationship to course of illness variables in panic disorder patients. In 32 panic disorder patients compared with 12 normal volunteer controls, plasma HVA was found to be significantly lower (47.1 vs. 61.1, $p < .01$) while MHPG, 5-HIAA, ACTH, and cortisol were not significantly different. As an alternative approach, he has studied the prevalence and incidence of anxiety disorders in patients with idiopathic Parkinson's disease. Nine of 24 patients (38%) had a clinically significant anxiety disorder. The severity of anxiety did not correlate with the history of parkinsonian impairment, the cumulative duration of L-DOPA exposure, nor the current dose of L-DOPA. These data suggest that anxiety disorders may commonly afflict patients with Parkinson's disease and should be considered in the medical evaluation and treatment of parkinsonian patients. In addition, they suggest that further attention be given to the role of dopaminergic and serotonergic systems in the etiopathology of anxiety and phobic disorders. These findings have also been highlighted by the recent Washington Post cover story indicating that Mohammed Ali suffers complete linguistic dysfunction in social situations but remains extremely fluent when interviewed by telephone.

Dr. Uhde and his collaborators (Cheryl Shea and others) have performed the first extensive double-blind, placebo-controlled clinical trial of several pharmacotherapies against cognitive-behavioral therapy (CBT) in the treatment of disabling social phobias. They demonstrated that all treatments were effective (including placebo) on most scales, although patients treated with alprazolam demonstrated a more rapid onset of action whereas

both phenelzine and CBT tended to show less relapse upon drug discontinuation. This study of 65 patients is the largest controlled clinical trial performed at the NIMH, and does much to answer critical questions about the relative efficacy and persistence of therapeutic efficacy across a variety of treatment conditions for this understudied but disabling psychiatric disorder. This is the first study to clearly document that drug therapy is an alternative treatment of an anxiety disorder previously considered as being purely psychogenic in origin. Current research will focus on clinical and biological factors which will identify those patients who preferentially respond to particular treatments. For example, preliminary evidence suggest that those social phobics who have normal GH responses to clonidine do not benefit from imipramine pharmacotherapy.

On a preclinical level, Dr. Uhde is studying behavioral and biochemical aspects of a colony of pointer dogs bred for normal and nervous behavior. "Nervous" dogs develop normally until age 8-12 months, when they begin to demonstrate marked behavioral impairments which resemble many aspects of pathological anxiety in man. They avoid human contact and freeze upon approach. Utilizing the selective benzodiazepine antagonist Ro-15-1788, preliminary evidence suggests that an excess of endogenous benzodiazepine receptor stimulation is not causally related to this syndrome in dogs. Evidence does, in contrast, suggest the possibility that adenosine receptor binding is abnormal in nervous dogs compared with litter-mate controls. An increase in binding of [^{3}H]-N^6-cyclohexyladenosine was observed in the hippocampus of nervous dogs and an increase in [^{3}H]-dipyridamole, an adenosine reuptake blocker, was found in the cerebellum.

Unit on Peptide Studies (Dr. David Rubinow, Chief)

Three types of projects are conducted within this functional group. First, studies in behavioral medicine are conducted in conjunction with a wide range of projects interdigitated into the Consultation-Liaison Service of the NIH which services all of the other Institutes within the Clinical Center. A prototypic example of the creative and novel interplay of this Service, clinical investigation, and basic neuroscience is elucidated by the studies of the endocrine effects of interleukin-2 (IL-2) administration in patients with metastatic tumors. Dr. Rubinow and his group elucidated the nature, timing, and incidence of the organic confusional psychosis associated with IL-2 treatment. An initial series of preclinical studies have suggested that alterations in the blood-brain barrier may not account for this syndrome, despite evidence of pathological edema occurring in patients following IL-2 treatment. A search for other possible mechanisms was initiated and a series of remarkable findings were uncovered. IL-2 was found to stimulate ACTH, beta-endorphin, and cortisol. Although repeat infusion the next day was associated with lesser degrees of stimulation, rechallenge following an interval of one week was associated with marked increases in the secretion of these substances. During a second course of IL-2 treatment, three to four months later, profound, 5-10-fold increases in ACTH, beta-endorphin, and cortisol levels were observed.

These data suggest that an extraordinary endocrine "sensitization" is occurring based on prior exposure to IL-2. Preliminary data suggest that those

patients showing the most vigorous "sensitization" during the second treatment show lesser degrees of subsequent tumor regression than those with more moderate endocrine responsivity. Further data are required to elucidate the possible clinical correlates of this sensitization as well as the underlying mechanism of this robust sensitization effect. These studies not only hold the promise of elucidating basic interrelationships between immune and endocrine systems in brain and man, but demonstrate an ideal paradigm for inter-Institute collaboration which should help to expand the frontiers of clinical neuroscience and, ultimately, lead to better treatment of both medical and psychiatric patients.

Dr. Rubinow published the first documented study of cognitive impairment in AIDs patients without evidence of CNS opportunistic infection. He continues to track longitudinal neuropsychological function in AIDs patients as well as in sero-positive AIDS-negative patients and controls. In addition, he has found preliminary evidence that high-dose interferon treatment of AIDS patients leads to the occurrence of significant clinical depression that is accompanied by cortisol hypersecretion. A series of other behavioral medicine protocols also include the investigation of the efficacy of psychomotor stimulants in depression associated with medical illness, evaluation of sleep EEG abnormalities and treatment response characteristics in patients with fibromyalgia, and investigations of conditioned effects on immune system function in association with chemotherapy.

A second major body of studies headed by Dr. Rubinow includes those of the menstrually-related mood disorders. Using pharmacological manipulations to alter the phase of the menstrual cycle (with RU-486, a cortisol and progesterone receptor blocker, used with and without human chorionic gonadotropin [HCG]), Dr. Rubinow has provided evidence that some women with premenstrual syndrome (PMS) experience their characteristic behavioral abnormalities (depression, irritability, anergia, inability to concentrate, etc.) in the context of an experimentally induced follicular phase of the cycle. As subjects do not know if they are receiving HCG (which will permit the continuation of normal ovarian function) or placebo (which will not prevent the RU486-induced luteolysis), they do not know if they are in the follicular or luteal phase following the RU-486-induced menses. Some women who experienced their PMS symptoms after the RU-486-induced menses were in the follicular phase. Thus, in these women, the PMS state appears to be linked to, rather than dependent on, reproductive endocrine changes during the luteal phase.

Dr. Rubinow's group has previously established the critical nature of longitudinal prospective monitoring of patients with menstrually-related mood disorder in order to arrive at a diagnostically homogeneous group of patients to enter into biological and pharmacological trials. Up to 50% of patients complaining of menstrually-related mood disorder were found, on prospective follow-up, not to have the disorder. This has been a potential reason for conflicting reports of biological and pharmacological findings in this disorder. Dr. Rubinow has not found significant alteration in sex hormones or endocrine substances which fluctuate with the menstrual cycle; in particular, estrogen and progesterone levels and patterns are not different in patients with menstrually-related mood disorders compared with controls. He has found

that a highly touted treatment for menstrually-related mood disorder, i.e., progesterone, is not significantly better than placebo in the treatment of this illness. He is now engaging in a series of other pharmacological trials in order to further clarify potentially effective treatment agents. Preliminary investigation of the administration of clonidine or placebo during the follicular and luteal phases has demonstrated acute mitigation of symptoms in four women given clonidine during the luteal phase. While efforts must be made to separate out nonspecific sedating effects from specific symptomatic improvement, this study provides a potential probe for exploring the pathophysiology (potentially alpha-2 noradrenergic) and treatment of menstrually-related mood disorders.

A trial of the antipanic agent alprazolam in PMS has also demonstrated a lack of systematic superiority compared with placebo. Similarly, a study of nalmefene, a long-acting opiate antagonist, revealed an exacerbation rather than improvement in symptoms. While clinically ineffective, this clinical investigation suggests the possibility of subtle alterations in opiate mechanisms of potential relevance to premenstrual mood dysfunction. The findings that up to 40% of women with menstrually-related mood disorders have abnormalities in their thyroid hormone axis has led to a controlled clinical trial of thyroid hormone supplementation in this syndrome.

A third group of studies involves neuroendocrine and peptide assessments in patients with manic-depressive illness. Dr. Rubinow and his group have pioneered in the findings of low somatostatin in the CSF of acutely depressed unipolar and bipolar patients which normalizes upon clinical improvement. He has also found a variety of important interrelationships between cortisol and various measures of neuropsychiatric dysfunction. In attempts to follow up on the possible regional localizations in brain of the finding of low somatostatin in CSF of depressed patients, he has studied regional concentrations of somatostatin in brains of suicide victims in comparison with accident victims. No significant differences were demonstrated in the amydala, hippocampus, or prefrontal cortex in the patient groups compared with controls.

Studies of salivary cortisol suggest that this readily obtainable measure may be a useful alternative strategy to obtaining blood by venipuncture during an endocrine challenge test such as with dexamethasone or CRH, making these procedures potentially available for outpatient and ambulatory studies. Salivary cortisol has also been been demonstrated to be increased following dexamethasone administration in patients treated with carbamazepine. However, repeated A.M. or P.M. monitoring of salivary cortisol on a longitudinal basis does not appear to provide a useful index or premonitory correlate of mood switching in moderate or rapidly cycling manic-depressive patients. The salivary cortisol measures have proven highly useful in studies of panic anxiety patients where significant increases have been demonstrated in association with anticipatory anxiety that develops after repeated caffeine administration.

Section on Psychobiology (Chief, R.M. Post, M.D.)

The acute and prophylactic clinical efficacy of the anticonvulsant carbamazepine continues to be documented in patients with lithium resistant affective

illness. Preliminary data suggest that many of the clinical markers associated with relatively poor response to lithium are associated with a better acute antimanic response to carbamazepine. These appear to include greater initial severity of mania, greater manic dysphoria, greater rapid cycling over the entire course of illness as well as increased cycling in the year prior to NIH admission, and a family history that is negative for affective illness in first-degree relatives.

Twenty-four of our patients on maintenance carbamazepine treatment used in conjunction with lithium carbonate have been followed for an average of four years with the range of follow-up extending up to ten years. Essentially all of these patients had been nonresponsive or poorly responsive to lithium carbonate and related traditional treatments of manic-depressive illness prior to the institution of carbamazepine. Total number of episodes per year was reduced approximately 50% in this group, and an index of illness which multiplies severity of functional incapacity and duration of an episode decreased by approximately 70%. Half the patients followed for more than two years showed a pattern of persistent suppression of episodes and affective morbidity on the illness index. However, the other half of the subjects showed a pattern of some loss of efficacy with reemergence of episodes during the second or third year of carbamazepine treatment.

These data suggest that patients followed during carbamazepine prophylaxis in the community may in some instances begin to show tolerance to carbamazepine's effects or a progression of the illness in the face of initially adequate pharmacotherapy. Preclinical models, such as the demonstration of conditioned tolerance to the anticonvulsant effects of carbamazepine on amygdala-kindled seizures described below, may be relevant to this development of loss of efficacy. Manipulations aimed at preventing or reversing this occurrence will be studied. Nonetheless, it is clear from our clinical studies and a rapidly growing international literature that carbamazepine is proving to be an effective alternative or adjunctive treatment in the management of a substantial subgroup of lithium-nonresponsive manic-depressive patients.

We have also conducted a series of clinical investigations of the carbamazepine-lithium combination. We have found in carbamazepine-nonresponders that lithium potentiation produces rapid antidepressant effects in 53% of patients studied. [Six of seven inadequately responsive manic patients showed a slower onset of antimanic response to lithium potentiation as well.] The selective rapid onset of lithium potentiation of carbamazepine in depression is consistent with a wide clinical literature indicating lithium potentiation of a variety of ineffective antidepressant modalities and suggests, because of its rapid onset, that this potentiation may be occurring by unique mechanisms.

In addition to the efficacy of the lithium-carbamazepine combination, a variety of clinical laboratory interactions have been noted. While carbamazepine alone produces significant decreases in granulocytes, adjunctive treatment with lithium not only normalizes this effect, but increases granulocytes above baseline. Recent preclinical data from Gallichio (1988) have demonstrated that carbamazepine suppresses bone marrow colony-stimulating

factors in a dose-dependent fashion and that lithium at therapeutic concentrations is able to override this effect. Thus, lithium may emerge as an important counter to the benign leukopenia of carbamazepine, but does not appear to be an agent that would be effective in the treatment of the idiosyncratic and rare hematological problems of agranulocytosis or aplastic anemia. While both lithium and carbamazepine decrease levels of T_4, free T_4, and T_3, only lithium increases levels of TSH. When the two drugs are used in combination, circulating thyroid hormone levels are decreased in an additive fashion but TSH levels are no further increased than they are with lithium alone. These data suggest that lithium and carbamazepine are interacting at different levels of the thyroid neuroaxis. In contrast to lithium carbonate, which induces clinical hyperthyroidism in a small but substantial number of patients, carbamazepine is virtually without this side effect. On the contrary, the degree of decrease in T_4 and free T_4 appears, paradoxically, associated with better degrees of antidepressant response. These data also may be pertinent to the findings discussed below that rapid cycling patients have relatively higher indices of free T_4 and T_4 than nonrapidly cycling patients.

While clinical levels of carbamazepine have not been correlated with degree of therapeutic response, some evidence suggests that carbamazepine's metabolite, its 10,11-epoxide, may exert anticonvulsant effects and be clinically useful in the treatment of trigeminal neuralgia. We are conducting a clinical trial to study the psychotropic efficacy of the 10,11-epoxide metabolite and preliminary data is supportive of its efficacy.

Response to other anticonvulsant agents is also being explored in patients with refractory manic-depressive illness. We have found that some patients respond to carbamazepine but not valproate and vice-versa. We have also identified several patients with inadequate responses to carbamazepine who have responded well to the combination of lithium and valproic acid. Another anticonvulsant, clonazepam, which is also being evaluated, exerts its actions selectively at the central-type benzodiazepine receptor, which is associated with a chloride channel. In contrast, carbamazepine appears to act through the peripheral-type benzodiazepine receptor, which has been associated with a calcium channel. Thus, it is possible that anticonvulsants with different mechanisms of action will show a different profile of clinical efficacy in patients with manic-depressive illness.

Several of these agents appear to be distinctly clinically useful in the treatment of lithium-refractory bipolar patients, particularly rapid cyclers. In an effort to identify clinical and biological markers of response to lithium vs. carbamazepine with Dr. K. Denicoff, we have initiated an outpatient clinical study of lithium vs. carbamazepine in a placebo-controlled, double-blind, crossover design. Patients will be randomized to lithium or carbamazepine in the first year, crossed over in the second year, and then switched to the lithium-carbamazepine combination the third year. In this fashion, we hope to identify possible predictors and correlates of clinical response so that patients can be more readily matched to their drug of choice from the outset of the clinical trial rather than resorting to an extended period of sequential empirical observation.

Carbamazepine appears to exert important effects through $GABA_B$ (baclofen)-type mechanisms that are implicated in its antinociceptive actions. Terrence et al (1983) reported that the inactive isomer of baclofen, d-baclofen, reversed the antinociceptive effects of both the active isomer, l-baclofen, and carbamazepine in an electrophysiological model of trigeminal neuralgia. In contrast, Dr. S.R.B. Weiss has shown that d-baclofen is unable to reverse the anticonvulsant effects of carbamazepine on amygdala-kindled seizures. These differential data implicating $GABA_B$ effects of carbamazepine in trigeminal neuralgia, but not in epilepsy, leave very much at issue whether the psychotropic effects of carbamazepine in manic-depressive illness are related to $GABA_B$ mechanisms. On this basis, and other preclinical data of Lloyd and collaborators (1987) suggesting that virtually all antidepressant treatments upregulate $GABA_B$ receptors in frontal cortex in rodents, we initiated a clinical trial of l-baclofen in acute depression. Preliminary data in a small number of patients suggest that this agent is, so far, an ineffective treatment.

In contrast to the anticonvulsant and antinociceptive effects of carbamazepine which appear acutely, the antimanic and antidepressant effects of carbamazepine appear to lag several weeks before onset of maximum clinical efficacy. This suggests that the mechanisms of action associated with chronic carbamazepine administration may be more closely related to its psychotropic effects in manic-depressive illness than its antinociceptive or anticonvulsant effects. In collaboration with Dr. S.R.B. Weiss, we have elucidated a seizure model that requires chronic carbamazepine treatment in order to demonstrate efficacy. In this fashion it may be possible to use a seizure model to elucidate mechanisms related to psychotropic drug action, at least in terms of the requirement for a lag in onset. In particular, we have elucidated that chronic but not acute carbamazepine blocks the development of lidocaine- and cocaine-kindled seizures. In addition, acute intermittent administration is without effect in these same seizure models where chronic oral administration is effective. These data suggest that different biochemical mechanisms may be effected by chronic as opposed to intermittent carbamazepine administration, which may also be relevant to carbamazepine's psychotropic properties.

Using carbamazepine in the developmental phase of amygdala-kindled seizures, we have elucidated the novel phenomenon of conditioned inefficacy to an anticonvulsant agent. In contrast to local anesthetic-induced kindling, where carbamazepine is effective in blocking the early developmental stages of pharmacological kindling, carbamazepine is unable to block the development of electrical kindling elicited from the amygdala in the rodent. However, once the animals are fully kindled and demonstrate completed kindled seizures, they are highly responsive to the anticonvulsant effects of carbamazepine. We have demonstrated conditioned inefficacy to carbamazepine by treating two groups of rats either with carbamazepine administered immediately prior to amygdala-kindling, during seizure development, or immediately after the kindling stimulation. In animals that received carbamazepine prior to each stimulation, unresponsiveness to carbamazepine is maintained during the period when they have been fully kindled (conditioned inefficacy) and should be responsive. In contrast, animals receiving carbamazepine after each kindling stimulation during the developmental phase are highly responsive to the anticonvulsant effects of carbamazepine when they are administered the drug before

stimulation. These data suggest that animals kindled in the presence of carbamazepine will subsequently show nonresponsiveness to this drug at a time when naive animals or animals treated with equal amounts of carbamazepine at an irrelevant time-point after kindling, are highly responsive. This conditioned inefficacy can be partially reversed by a period of treatment with carbamazepine after seizures, or by a period of kindling without carbamazepine.

A related phenomenon of conditioned tolerance has also been demonstrated by Dr. Weiss. Once animals enter the stage of completed kindled seizures and become responsive to the anticonvulsant effects of carbamazepine, repeated pairing of drug pretreatment with the kindled stimulation eventually leads to the loss of efficacy of carbamazepine and seizures reemerge. This tolerance to carbamazepine can be reversed by a period of treatment when the drug is given after the seizure occurs or by a period of kindling without drug. In contrast, merely waiting an extended period of time (up to three weeks) or treating animals with carbamazepine in the absence of kindling stimulation is not sufficient to reverse the conditioned tolerance. These phenomena of inefficacy or tolerance are not related to blood levels of carbamazepine, as control animals are treated with the same doses. Furthermore, animals treated with higher doses of carbamazepine (25 mg/kg instead of 15 mg/kg i.p.), if anything, demonstrate faster development of tolerance compared with lower doses. The conditioned tolerance appears to be drug-specific, since carbamazepine-tolerant animals are still responsive to the anticonvulsant effects of a benzodiazepine such as diazepam.

The phenomenon of conditioned tolerance may have implications not only for patients with seizure disorders, but also for those with trigeminal neuralgia, many of whom develop increasing nonresponsiveness to carbamazepine after initial periods of efficacy. As described above, some of our manic-depressive patients also appear to be developing tolerance to the psychotropic effects of carbamazepine with increasing emergence of affective episodes. The preclinical data raise the possibility that manipulations capable of retarding or reversing the conditioned tolerance may be appropriate to neuropsychiatric patients who have demonstrated loss of efficacy to the effects of carbamazepine. In addition to their potential clinical relevance, the findings of conditioned inefficacy and conditioned tolerance raise basic questions regarding the conditional molecular mechanisms that are involved in such a phenomenon. Preclinical studies will be directed at uncovering these mechanisms which suggest that phenomena relevant to conditioning and learning may impact on pharmacological responsivity.

In light of carbamazepine's profile as the most potent anticonvulsant in inhibiting fully developed amygdala-kindled after-discharges and seizures, and its role in the treatment of complex partial seizures, we have explored a variety of measures of temporal lobe and limbic dysfunction in patients with affective disorders. Preliminary data collected in association with Drs. P. Hauser and L. Altshuler suggest that patients with primary affective illness have decreased temporal lobe to cerebral area ratios as measured by magnetic resonance imaging (MRI) techniques. Right temporal lobe to cerebral ratios in controls decreased as a function of age, while this was not significant in patients with affective disorders. In contrast, patients with affective

disorders had a significant relationship between decreasing temporal lobe to cerebral area ratios and duration of illness. These findings are of interest in their own right in relationship to affective illness, but also suggest that the similar relationships reported in patients with schizophrenia may not be specific to diagnosis, and the implications of these findings require further clinical investigation. Dr. Altshuler has examined mood and anxiety patients with complex partial seizures and has demonstrated that those with a left-sided seizure focus have higher levels of depression compared with those with a right-sided focus or compared with controls studied in collaboration with Dr. O. Devinsky. A pharmacological probe of limbic system function with the local anesthetic procaine has been demonstrated to produce increases in fast EEG frequencies over the temporal lobes (studied with Dr. R. Coppola). This effect has been correlated with degree of dysphoria. However, clinical and EEG responses do not appear to be associated with degree of psychotropic response to the anticonvulsant carbamazepine in patients with borderline personality disorders studied in collaboration with Drs. R. Cowdry and D. Gardner. Procaine also induces a pattern of endocrine activation suggestive of release of corticotropin-releasing factor (CRF). In normal subjects and psychiatric patients, procaine induces dose-related increases in ACTH and cortisol and prolactin, but no significant change in growth hormone (studied with Dr. M. Kling). Preclinical studies of Rivier and Vale and Calogero, Gold et al. suggest that the local anesthetics may be directly releasing CRF in several different tissue preparations.

In accord with the lack of predictive effects of procaine in relationship to carbamazepine response in patients with borderline personality disorders, we have also not observed a relationship of baseline psychosensory symptoms (i.e., those often reported by patients with temporal lobe epilepsy) and degree of clinical antimanic or antidepressant response in patients with affective disorders. These data, in conjunction with PET scans utilizing deoxyglucose, which show, if anything, temporal lobe hypometabolism in patients with affective disorders, indicate that there is very little direct clinical evidence to support a relationship of temporal lobe epileptiform activity and response to the anticonvulsant carbamazepine.

We have developed a systematic life-charting system to follow the longitudinal course of manic-depressive illness and relate this evolving clinical course to various biological measures. An example of one such recent finding involves the thyroid axis. Alterations in thyroid function have long been postulated to be significant for the induction of changes in mood and behavior, and half a century ago Gjessing treated patients with periodic catatonia with high doses of thyroid hormone with some success in ameliorating their rapid or continuous cycling pattern. These and related data led to the suggestion that rapid cycling illness may be associated with relative hypothyroidism. In contrast, in a series of more than 100 patients, we have documented that rapid cycling is associated with significantly higher levels of T_4 and free T_4, and that patients with initially higher basal levels of T_4 show a better antimanic response to carbamazepine. These thyroid data were collected after an extended period of medication-free evaluation during which there seemed to be progressive changes in thyroid function. During the last thyroid assessment after an average of eight weeks medication-free compared with the first

thyroid assessment after an average of two weeks medication-free, there were significant decreases in T_3 and increases in T_4 and free T_4, perhaps consistent with the development of euthyroid sick syndrome with lesser conversion of circulating T_4 to T_3. This pattern was observed significantly more in rapid cycling patients and in those with higher levels of circulating glucocorticoids. These data, taken in conjunction with the data mentioned above that both lithium and carbamazepine suppress rather than enhance thyroid indices and that carbamazepine's efficacy in acute depression is positively associated with the degree of decrease in T_4 and free T_4, suggests that conventional wisdom that rapid cycling is associated with relative hypothyroidism needs to be reevaluated. Perhaps previous findings were confounded by lithium co-treatment. Clearly, the complex interrelationships with thyroid function in affective illness deserve careful evaluation in relationship to both their clinical and theoretical impact.

Dr. Weiss has uncovered novel findings in the field of chronic psychomotor stimulant administration. She has demonstrated that the increased behavioral responsivity upon administration of cocaine is environmental-context-dependent, related to the degree of similarity of pretreatment and test environments, and dependent on the expression of cocaine-induced behaviors themselves. For example, if cocaine-induced hyperactivity is blocked with high doses of neuroleptics or diazepam, animals will not demonstrate increased responsivity upon subsequent rechallenge. In contrast, once cocaine-induced behaviors are manifest, even high doses of neuroleptics administered prior to the day-2 challenge dose are insufficient to block the sensitization effect. Thus, it appears that neuroleptics block the development but not the expression of cocaine-induced behavioral sensitization. In contrast, diazepam and clonidine appear capable of blocking both the development and the expression of cocaine-induced behavioral sensitization. These preclinical data suggest that the differential responsivity to neuroleptics is a function of phase of development of sensitization and may be a useful animal model for neuroleptic nonresponsiveness in some psychotic states. A variety of clinical data reviewed by Wyatt does, in fact, support the notion that early treatment of psychotic patients may be more effective than treatment initiated later in the illness, when psychotic symptoms have been fully expressed. Such data may be comprehensible from the perspective of the model of differential neuroleptic responsiveness in cocaine-induced behavioral sensitization.

While low doses of cocaine produce increases in behavioral responsivity upon repeated administration (behavioral sensitization), repeated high dose cocaine administration leads to a pharmacological kindling effect. That is, animals begin to develop seizures to a dose of cocaine which was previously subconvulsant. Dr. Weiss has demonstrated that chronic administration of cocaine will block the development of both lidocaine- and cocaine-induced pharmacological kindling. In addition, chronic carbamazepine blocks the lethality associated with cocaine-induced seizures. However, acute carbamazepine administration is without effect and may even increase lethality. Carbamazepine does not block the development of cocaine-induced behavioral sensitization but does block the pharmacological kindling effect.

These and other data suggest that behavioral sensitization may be mediated by dopaminergic mechanisms (that are not affected by carbamazepine) while the pharmacological kindling effect is mediated by the local anesthetic properties of cocaine (which are blocked by carbamazepine, possibly through common actions at type-2 sodium channels). Fifty percent of callers to a Cocaine Hotline (Washton & Gold) have reported the experience of cocaine-induced panic attacks. We have postulated that cocaine-induced panic attacks develop in a kindling-like fashion and that carbamazepine would be effective in preventing their development. Preliminary data suggest that carbamazepine, while it is not effective in the treatment of primary panic disorder, may be of use in the treatment of the cocaine-related syndrome. We are initiating studies on the possible mechanisms underlying the evolution of cocaine-related anxiety disorders and related psychiatric disturbances.

The preclinical data on behavioral sensitization and pharmacological kindling strongly suggest that repeated cocaine administration may carry several different types of hidden dangers. In particular, these data suggest that behavioral and physiological toxicities may progressively emerge with chronic administration, even of the same dose, over time. These data, taken in conjunction with other data suggesting tolerance to the euphoria-inducing properties of cocaine leading to dose escalation, may help explain the progression of some psychiatric and physiological dysfunction with chronic cocaine administration. We are also currently attempting to elucidate mechanisms of post-ictal death following cocaine administration. In contrast to lidocaine seizures, which are well tolerated even following repeated episodes, cocaine-induced seizures are usually lethal in the rodent after the first or second seizure episode.

Since cocaine appears to release CRH, and CRH has previously been demonstrated to induce limbic seizures, Dr. Weiss and colleagues are currently exploring the possible role of CRF in cocaine-induced behavioral and convulsive syndromes and their evolution. This series of studies on chronic cocaine administration should prove valuable in understanding the evolution of behavioral and physiological toxicities to cocaine in man, but also as models for affective, anxiety, and schizophreniform syndromes since chronic cocaine administration has been associated with a panoply of neuropsychiatric syndromes in man. Understanding the mechanisms underlying cocaine-induced behavioral pathology may, in this fashion, ultimately help understand mechanisms underlying endogenous psychoses and primary anxiety disorders.

Unit on Behavioral Pharmacology, Agu Pert, Ph.D., Chief

Dr. Pert has utilized *in vivo* microdialysis techniques to study effects of psychoactive drugs on dopamine function in anesthetized animals. The technique is being modified so that it can be utilized in awake behaving animals as well. He has utilized this technique to study the effects of acute and chronic cocaine administration on dopamine and its metabolites in striatum, nucleus accumbens, and frontal cortex of rats. Various concentrations of cocaine (0.01-10mM) were introduced through the dialysis probes and alterations in dopamine and dopamine metabolites were measured in the dialysate with HPLC methodology. When cocaine hydrochloride is introduced into the dialysis probe,

it produces dramatic increases in dopamine in a dose-related fashion in striatum and nucleus accumbens, but only minimal and nondose-related changes are observed in the frontal cortex. Dr. Pert found that dopamine responses to all concentrations of cocaine were greater in magnitude in the striatum than those in the nucleus accumbens. These findings indicate that there are differential dopamine responses to cocaine in three dopamine-rich brain areas and suggest the involvement of different mechanisms. One possibility is differences in dopamine uptake mechanisms in these areas, because, for example, it is known that the nucleus accumbens has only low affinity dopamine uptake sites, while the striatum has both low and high affinity sites. When animals are treated with cocaine (30 mg/kg i.p.) for seven days, there is no apparent change in the dose-response relationship for dopamine in the dialysate, suggesting that cocaine-induced behavioral sensitization involves mechanisms that are postsynaptic to dopamine terminals.

There is considerable controversy regarding the effect of opiates on dopamine function. Early studies suggest that opiates block dopaminergic function, while more recent ones have indicated that opiates may actually enhance dopamine release. In studies comparing active and inactive isomers of morphine, it was found that only the active enantiomer of morphine enhanced dopamine release in the striatum using unilateral microdialysis probes. Injections of morphine into the striatum, however, produced a stereospecific decrease in dopamine release in the ipsilateral striatum. Thus, method and route of injection may be critical in effecting differential release of dopamine by opiate active compounds. Mu-type opiates appear to have a dual and opposite action on release of nigrostriatal dopamine.

The pharmacology of kappa-opiate receptor agonists has been extensively explored. Much effort has been directed to the development of nonaddictive opiate analgesics, and it was originally hoped that benzomorphan analogues that selectively interact with the kappa-opiate receptors might fulfill this need. U50,488 is a highly selective kappa agonist, which upon intracerebral injection produces marked effects on locomotor activity, the active (s,s) isomer is much more potent than the inactive enantiomer (r,r). U50,488 produced an initial dose-dependent decrease in locomotor output which was followed by excitation. No tolerance to the locomotor depressant effects was observed following chronic administration, while the excitatory effects increased with repetitions. Neither MR-2266 nor naloxone antagonized the excitatory effects of U50,488. 6-Hydroxydopamine lesions of the nucleus accumbens also failed to alter the locomotor stimulatory effects. The effects of U50,488 are different following systemic compared with central administration and do not appear to involve mesolimbic dopamine.

(s,s)-U50,488 (the active enantiomer) increased reaction latencies in the hotplate, paw pressure, and tail-flick tests. In contrast, (r,r)-U54,88 (the inactive enantiomer) did not alter the reaction times in any test. The effects of the active enantiomer were relatively resistant to antagonism by either naloxone or MR-2266. Upon intracerebroventricular injection, both active and inactive enantiomers were highly effective on hotplate and paw pressure test, however. Nor-BNI did not antagonize the effect of (s,s)-U50,488 in any

test. These findings suggest that kappa agonist antinociception does not involve superspinal mechanisms, but is probably mediated either peripherally or at a spinal level.

Another area of current importance both in relationship to drug abuse and also as a potential model for schizophreniform psychosis is the behavioral and biological effects of the psychotomimetic compound phencyclidine (PCP). PCP is a powerful psychotomimetic substance that produces psychopathological effects that mimic the primary symptoms of schizophrenia. Many of its effects have been thought to involve dopaminergic mechanisms. In previous studies, Dr. Pert and colleagues have identified the substantia nigra as an important focus for the behavioral actions of PCP. In order to further define the functional outputs of the nigrostriatal system that are activated by PCP, rats were injected with 25 nM of PCP into the substantia nigra and intravenously injected with 100 μCi/kg of 2-deoxyglucose (2-DG). Intranigral PCP was found to produce profound rotational behavior contralateral to the injection. This behavior was accompanied by increases in uptake of deoxyglucose in the habenula, media forebrain bundle, superior olive, caudate putamen, striatal fundus, pedunculopontine trigeminal nucleus, and the cerebellum. Decreased uptake was observed in the cingulate cortex, medial thalamus, periaqueductal gray, substantia nigra, retrorubral field, and the superior colliculus. Decreases in substantia nigra metabolic activity resulted in both increases and decreases in metabolic activity of the mesencephalic and diencephalic structures. Since it was previously shown with lesioning techniques that only structures caudal to the substantia nigra are critically involved in mediating the effects of intranigral PCP, it appears that alterations in metabolic activity of mesencephalic and pontine regions are related to the motor asymmetrics induced by this manipulation, while alterations in metabolic activity of more rostral structures are related to other pharmacological effects induced by this manipulation.

In addition to PCP, MK801, as well as SKF10047, also appear to have similar psychotomimetic properties. Each appears to exert noncompetitive antagonism of glutamate receptors of the n-methyl-d-aspartate type (NMDA). Rats were injected with these compounds followed by injection 30 minutes later with (14^{C})-2-deoxyglocose. Significant increases in metabolic activity were found in the anterior and posterior cingulate cortex, anteroventral, ventromedial, and posterior thalamic nuclei, dorsal and ventral hippocampus, and substantia nigra zona reticulata following MK801. PCP also enhanced metabolic activity in the anterior and posterior cingulate and the substantia nigra zona reticulata as well as the caudate nucleus. Unlike MK801, however, PCP decreased metabolic activity in the thalamic nuclei. In general, both enantiomers of SKF10047 reduced the metabolic activity of a variety of structures, including the hippocampus, the majority of the thalamic nuclei, and the substantia nigra. It is clear that these noncompetitive NMDA antagonists have vastly different consequences on brain metabolic activity, even though all seem to share some common psychotomimetic effects.

Dr. Pert has also studied brain mechanisms involved in the immunosuppressant effects of morphine, and has previously implicated the periaqueductal gray (PAG). Injections of morphine into the PAG produced a significant suppres-

sion of natural killer cell activity and T-cell proliferation in response to mitogen, while injections into a variety of other brain structures were without effect. Stimulation of the ventromedial aspects of the PAG produced profound aversive reactions in animals which are also accompanied by a significant suppression of natural killer cell activity. Thus, it appears that PAG may represent an important integrative region for the mediation of aversive consequences of exogenous stressors on immune function.

The functional outflow of the nucleus accumbens has been studied as it represents a critical focus for the motor activating effects of psychomotor stimulants as well as a critical area for rewarding properties of these drugs. Injection of amphetamine (15 nM) into the nucleus accumbens produced significant increases in locomotor activity which were acccmpanied by alterations in the uptake of 2-DG in a variety of structures. Increases in uptake were seen in the ventral pallidum, nucleus of the diagonal band, PAG, retrorubral field, red nucleus, substantia nigra (zona reticulata), pontine reticular nuclei, and ventrotegmental area. Decreased uptake was seen in frontal parietal cortex, red nucleus of the striaterminalis, medial thalamus, and lateral habenula. Thus, there appears to be a pattern in which the most prominent changes were found in the terminal areas of the nucleus accumbens efferent projections. Alterations seen in mesencephalic and thalamic regions are probably related to the locomotor stimulatory effects of amphetamines.

Unit on Neurochemistry, Paul J. Marangos, Ph.D., Chief

Several major studies of quantitative autoradiography of the adenosine system were completed in collaboration with Drs. Daval and Deckert. The effects of transient cerebral ischemia on adenosine receptors were studied in the gerbil brain. It was shown that CHA (an adenosine agonist) has marked protective effects in cerebral ischemia, indicating that adenosine agonist properties may ultimately prove important in the treatment of stroke patients. Following ischemia, adenosine receptors decreased drastically in several brain areas, mainly in hippocampus. The decrease in adenosine A1 receptors was associated with a parallel decrease in G-protein (labeled with ^{3}H-Gpp(NH)p) and adenylate cyclase (labeled with [^{3}H]-forskolin). CHA preserves the CA-1 pyramidal cells in the hippocampus of gerbils subjected to 20 minutes of ischemia.

Another focus of investigation has been the elucidation of the mechanisms of action of carbamazepine as they relate to adenosinergic mechanisms. Paradoxically, even though caffeine and carbamazepine have opposite physiological profiles, both have been demonstrated to upregulate adenosine receptors following chronic administration. To follow up on the findings and discern possible regional differences in the pattern of upregulation, Drs. Daval and Deckert performed autoradiographic studies. They found that virtually identical patterns of adenosine receptor upregulation were observed throughout most brain regions. In the first study to demonstrate functional alterations in [^{3}H]-forskolin binding following chronic drug administration, they found that both carbamazepine and caffeine also similarly increased binding to this moiety which is closely associated with adenylate cyclase function. Thus, it appears that both adenosine receptors and a measure of their functional subreceptor

apparatus (as reflected in [^{3}H]-forskolin binding) are upregulated by chronic administration of these two agents, further suggesting that carbamazepine, paradoxically, possesses adenosine antagonist properties.

Work in the laboratory has also focused on the possible mechanisms involved in behavioral sensitization and kindling. Studies with Drs. Nakajima, Daval, and Gleiter have focused on the proto-oncogene product c-fos. It has been demonstrated in brain that electroconvulsive seizures cause a rapid and transient expression of c-fos mRNA. Of particular interest in this study was the observation that even the ear-clip control animals (no ECS) manifested a slight increase in c-fos expression, suggesting the possibility that activation of this oncogene may be stress- as well as seizure-sensitive.

Most recent studies have documented a dose-response relationship of caffeine to c-fos expression in mouse brain. Interestingly, caffeine-induced increases in c-fos can be blocked by diazepam and the relatively inactive receptor antagonist RO15-1788. These and studies in other laboratories suggest the possibility that c-fos may represent a novel marker of neuronal activity and be useful in mapping neuronal activity in brain. As an oncogene, it may well affect various aspects of neuronal function in a long-lasting fashion, and thus is a potentially important substance to further explore in relationship to the long-lasting effects of sensitization and kindling.

It is well established that adenosine inhibits calcium-dependent release of many neurotransmitter substances. Calcium channel mechanisms have been of interest both in their own right and as they might relate to adenosinergic effects. Until the present, studies of calcium channel binding have utilized agents that act at the L-type, which predominates in muscle. Recent data suggests that a 27-amino acid toxin called conotoxin may bind to neuronal or N-type calcium channels, with weak but relative selectivity. This N-type channel has been shown to be involved in the neurotransmitter release and therefore may be particularly important in relationship to neuronal activity and seizure induction. A receptor binding assay for conotoxin has been developed using (^{125}I)conotoxin. Initial studies indicate different regional effects of ECS and lidocaine seizures on (^{125}I) conotoxin binding. Acute but not chronic ECS decreases both nimodipine and conotoxin binding in cortex. Acute and chronic lidocaine seizures decrease hippocampal binding of nimodipine without affecting conotoxin. Chronic, but not acute ECS also decreased binding of [^{3}H] phorbol-12,12-butyrate, suggesting a reduction in membrane bound protein kinase C.

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The Clinical Psychobiology Branch

Thomas A. Wehr, M.D.

The research program of the Clinical Psychobiology Branch continues to focus on a search for causes of depression and manic-depressive illness and on the development of new types of treatment for these disorders. In particular, we have attempted to understand how 1) changes in the seasons and 2) changes in the timing and duration of sleep can cause and can terminate mania and depression. Knowledge of the mechanisms whereby seasons and sleep influence clinical state almost certainly would lead to a better understanding of the pathogenesis of affective illness and to novel ways to treat and to prevent its episodic recurrences.

The fact that depression and mania can recur at certain seasons of the year is important because it suggests that these states may be caused by changes in physical aspects of the environment that vary with the seasons. So far, we have discerned three seasonal patterns: 1) winter depression with summer hypomania; 2) summer depression with winter hypomania; and 3) winter and summer depression with spring and fall hypomania. Much of our original work in this area concentrated on winter depression, but recently we have begun to investigate a substantial number of patients in the second and third categories. In our work with winter depression we repeatedly found that bright artificial light improves the condition, and we therefore infer that seasonal changes in natural light control the course of illness in these cases. We are just beginning to investigate causes and treatments of summer depression. Our preliminary evidence suggests that environmental temperature is capable of controlling clinical state in these patients. The capacity of heat to induce depression and cold to induce hypomania, if confirmed, may correspond to their capacities, respectively, to ennervate and stimulate normal individuals.

Total sleep deprivation, partial sleep deprivation in the second half of the night, and shifting the timing of sleep several hours earlier than usual, are all capable of improving depression on the one hand and causing mania on the other. Our research has shown that being awake or asleep in the latter half of the night appears to be critical for these effects. During this period REM sleep propensity is greatest; therefore the clinical effects of sleep deprivation might be due to REM sleep deprivation. Several avenues of research in our branch have led us to propose that a function of REM sleep may be to selectively warm the brain during the hypothermia which occurs in the rest of the body during sleep (see below). In depression body and brain temperatures are abnormally high during sleep. In addition, REM sleep occurs abnormally early in sleep and is abnormally intense. Thus, increased brain temperature during sleep could be related to this increased REM activity, and sleep deprivation may prevent this brain heating. In this regard, it is interesting to note that our studies of mechanisms of season- and sleep- induced mood changes are converging on temperature-regulating mechanisms. It is also interesting to note that, like winter and like sleep deprivation, antidepressant drugs lower body and brain temperature.

We have extensively investigated the effects of an antidepressant drug, clorgyline, on the activity-rest cycle in experimental animals, and find another kind of convergence, between effects of the drug and effects of light. In animals clorgyline induces behavioral effects analogous to those which occur in patients treated with the drug, and, as in patients, it takes two to three weeks for these effects to become evident. Many of these effects of clorgyline can also be induced by lesions of the lateral geniculate nucleus, a way-station in a pathway that mediates effects of light on the hypothalamus. The possibility that visual pathways to the hypothalamus mediate the antidepressant effects of phototherapy and antidepressants drugs is being explored in ongoing experiments.

All of this research suggests that depression and mania may be triggered and treated by changes in environmental temperature and light, and that some antidepressant drugs may act via their effects on nerve pathways that mediate the actions of temperature and light on the organism. From this research new fields, which we might call environmental psychiatry and environmental pharmacology, are emerging.

The seasonality of affective illness and the sensitivity of the illness to environmental factors may be clues to its nature: it might be a disorder of systems that mediate the organism's adaptations to changes in the physical environment. Consistent with this hypothesis is the fact that many animals, in their normal adaptations to changes in the physical environment, exhibit behavioral and physiological adjustments that are similar in kind and degree to those exhibited by manic and depressed patients. For example, at certain times some animals eat more, gain weight, become lethargic, withdraw from the environment and sleep much of the time; at other times they become

more active, interact more with their physical and social environment, have increased sexual drive, and lose weight.

There are also many parallels between the time course of these changes in animals and the time course of changes in affective patients. The changes are reversible and recurrent and may recur episodically or cyclically. Cyclic recurrences can be synchronized with seasonal cycles in the environment, or they can be independent of seasonal cycles (when animals are removed from their natural environment). The changes can be precipitated, sometimes quite rapidly, by changes in environmental factors, such as light and temperature.

These changes in animals should not be taken too literally as models of affective illness, but an understanding of their biological function may help to explain the nature of affective illness. With regard to their function, many of these changes in animals, in spite of their diversity, can be understood as strategies for managing the energy economy of the organism in the face of changing external challenges to energy balance. The hypothalamus is the focal point of a neural network that integrates the actions of energy-balance mechanisms and coordinates them with changes in the environment. Through nerves and humoral factors it monitors and regulates exposure to external factors that affect energy balance, such as light, temperature, and nutrients, and it monitors and regulates internal factors that help to control energy balance, such as appetite, metabolism, weight, physical activity, drive to interact with the physical and social environment, sleep and body temperature. The autonomic nervous system, and the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes, all of which have been implicated in affective illness, are important mediators of energy homeostasis. The hypothalamus also regulates and coordinates the timing of changes in these factors via pacemakers that generate daily and seasonal rhythms. If affective illness is a disorder of the network of physiological systems that regulates energy balance, then it is probably these structures which are affected.

Studies On Thermoregulatory Functions of Normal Sleep
Thomas A. Wehr, M.D.

Little is known of the biological functions of sleep and of the stages of sleep, such as slow-wave sleep and REM sleep. There is some agreement that sleep may serve to conserve energy, in a manner analogous to torpor, by inhibiting behavioral responses to environmental stimuli and by lowering the regulated level of body temperature and metabolism. In a natural environment such repeated small savings in energy expenditure could enhance an animal's chances of survival in environments with marginal energy resources. Work of other investigators has focused mainly on energy conserving aspects of slow wave sleep. Slow wave sleep appears to occur in association with a thermolytic, or temperature-lowering mechanism. Animals enter hibernation (a state of profound hypothermia) through slow-wave sleep, and, in human beings, heating of the head during sleep induces slow-wave sleep, even at the end of the sleep period when slow wave is normally absent.

There has been little investigation of the possible thermoregulatory functions of REM sleep. In fact, current dogma states that thermoregulation is suspended during REM sleep. Considerable energy is expended by the brain during REM sleep, and it seems unlikely that this expenditure serves no useful purpose. We hypothesize that the function of the marked increase in energy expenditure by the central nervous system during REM sleep is to generate heat locally in the brain and the eyes in order to maintain CNS temperature within acceptable limits while the rest of the body cools during sleep. Thus, according to this hypothesis, REM sleep is a form of selective brain thermogenesis. CNS thermogenesis during REM sleep is accomplished through 1) increased metabolism and 2) increased eye muscle tone and movement. Ultimately, the purpose of brain temperature maintenance during sleep may be 1) to facilitate rapid arousal from sleep and/or 2) to protect CNS tissue in homeotherms from functional or structural impairment during the considerable body cooling which occurs when they sleep in a natural environment.

There are many connections between homeothermic temperature regulation and REM sleep. For one thing, REM sleep probably only occurs in homeotherms and may have co-evolved with homeothermy independently in mammals and birds. The duration of the REM non-REM cycle is highly correlated with an animal's size, or more specifically, with the animal's surface area to mass ratio, which determines the rate at which heat is lost from the body. Also, there is a close, inverse relationship between REM propensity and the daily rhythm in body temperature; REM sleep is greatest when body temperature reaches its minimum at the end of the sleep period. During

development, the amount of REM sleep is greatest at the beginning of life and declines as somatic thermoregulatory mechanisms mature.

In order to investigate possible thermoregulatory functions of REM sleep we have carried out 1) descriptive studies to document changes in somatic and CNS temperature during the different stages of sleep and 2) experiments in which thermal challenges are administered to the sleeping brain to test the hypothesis that REM sleep is a thermogenic mechanism for maintaining brain temperature during body cooling in sleep.

In our descriptive studies we found that rectal, forehead, eyelid and tympanic temperatures decline rapidly during slow wave sleep. We found that rectal temperature (a measure of somatic temperature) continues to decline, but forehead, eyelid, and tympanic temperatures (indirect measures of CNS temperature) rise during REM sleep. During voluntary rapid eye movements in awake subjects, forehead, eyelid and tympanic temperatures rise, as occurs in REM sleep. These observations are in accord with our model of selective brain thermogenesis during REM sleep.

In experiments currently in progress we are studying the effects of facial heating and cooling on REM sleep generating mechanisms.

Within the framework of the research conducted by this branch, any knowledge about biological mechanisms responsible for REM sleep would be relevant to the basic mission of investigating causes and developing new treatments for affective illness. There are many important connections between REM sleep and depression. Extensive research has shown that certain manifestations of REM sleep are excessive in depressed patients, and most effective treatments for depression suppress these manifestations of REM sleep. Other evidence indicates that temperatures during nocturnal sleep are abnormally elevated in depressed patients, and that these elevations may be responsible for some of the endocrine abnormalities observed in depression. Our sleep deprivation experiments and our studies of seasonal influences on depression suggest that excessive heating may induce depressive symptoms. If our hypothesis that REM sleep promotes CNS thermogenesis is correct, then its putative capacity to trigger depression might be related to its capacity to heat the brain.

Effects of Sleep and Sleep Deprivation On Mood
Thomas A. Wehr, M.D.

Total sleep deprivation for one night induces temporary remissions in sixty percent of patients with major depression; it can also induce mania in bipolar patients. These observations have practical implications for the management of affective illness. For example, some patients' depressions can be treated with sleep deprivation, and sleep disruption is sometimes an identifiable and preventable cause of mania. Knowledge of biological mechanisms of sleep deprivation would undoubtedly increase our understanding of the causes of depression and mania and lead to new types of drug treatments for depression and mania. We have conducted a series of experiments whose purpose is to identify biological mechanisms of the antidepressant effects of sleep deprivation by 1) determining those aspects of the sleep deprivation procedure which are responsible for its antidepressant effects, and 2) investigating the effects of sleep deprivation on biological variables, such as hormones and neurotransmitter metabolites, which might mediate its antidepressant effects.

We have already determined that 1) exposure to light at night is not necessary for the antidepressant effects of the procedure and 2) partial sleep deprivation in the second half of the night is much more effective than partial sleep deprivation in the first half of the night.

We found that sleep deprivation has little effect on levels of plasma melatonin, homovanyllic acid (HVA) or 3-methoxy, 4-hydroxyphenylglycol (MHPG). On the other hand, sleep deprivation increases plasma levels of thyrotropin (TSH) and cortisol and decreases plasma levels of prolactin (PRL) and growth hormone. The fact that sleep deprivation increases plasma levels of thyrotropin (TSH) is of particular interest because we found that nocturnal levels of TSH are deficient in depressed patients. The effects of sleep deprivation on TSH resemble effects of cold exposure. Therefore, in an ongoing experiment we are testing the hypothesis that the effects of sleep deprivation on mood and on TSH secretion in depressed patients depend on heat loss from the organism. According to this hypothesis sleep deprivation in a warm environment should be less effective than sleep deprivation in a cold environment. Patients and normal individuals are being sleep deprived on one occasion in a very warm environment (90° F), and on another occasion in a very cool environment (62° F). So far, we have found that heating of the environment increases

rectal temperature and decreases TSH levels. In the patients, antidepressant effects of sleep deprivation appear to be greater in the cool condition than in the warm condition. So far, these results are consistent with predictions based on our hypothesis, but the research must be extended to additional subjects to permit statistical evaluation of the results.

Causes and Treatments of Rapid Cycling Affective Disorder
Thomas A. Wehr, M.D.

Our longstanding interest in the relevance of biological rhythm research to affective illness led us to study the cyclicity of the illness. One very fruitful outcome of this interest was our description of various forms of seasonal affective disorder and their treatment with manipulations of light and temperature. Another fruitful outcome of this interest was our discovery that tricyclic antidepressants are capable of accelerating the cycles of affective illness, increasing the frequency of recurrences. This finding emerged from several years of observations of more than fifty patients with rapid cycling forms of affective illness.

One of the distinguishing features of affective illness is its tendency to remit and recur spontaneously and with increasing frequency. The course of bipolar illness is also characterized by a tendency for manic episodes to be immediately preceded or followed by depressive episodes, with no intervening normal period, and for mania to alternate with depression. In rapid cycling affective disorder these tendencies are so pronounced that mania and depression recur regularly and frequently with a continuous, circular course. Rapid cycling cases comprise about 15% of patients in lithium or affective disorder clinics, and they are difficult to treat.

In order to gain some insight into the causes and treatments of rapid cycling affective disorder we compared data obtained from rapid cycling and non-rapid cycling patients admitted to our research program since 1973. Since 1973 we had accumulated a large amount of information from prospectively longitudinal observations and structured clinical interviews on these patients' psychiatric and medical histories, family histories, course of illness, and responses to treatments. In our patients, rapid cycling was defined as four or more affective episodes per year that, in bipolar patients, followed a circular course at some time in the history of the illness.

Our findings suggest that rapid cycling affective disorder is phenotypically and genetically related to more typical forms of bipolar affective disorder. First, all the rapid cycling patients had met the RDC for bipolar affective disorder. Second, the ages at onset of affective illness in the rapid cycling patients were strikingly similar to those reported for non-rapid cycling bipolar patients. Third, in more than half the cases, patients who ultimately developed rapid cycling began their illness with a pattern of occasional, isolated episodes that resembled the typical course of illness in non-rapid cycling bipolar patients. Fourth, family histories revealed a high genetic loading for non-rapid cycling unipolar and bipolar affective disorder, similar to that seen in non-rapid cycling cases, and a small familial tendency for rapid cycling.

We found that there was a high prevalence of thyroid disease during lithium treatment in both rapid cycling and non-rapid cycling women. These findings are consistent with other reports of a high prevalence of hypothyroidism in women over 40 years of age who have been treated with lithium. These findings contrast with earlier reports of a higher prevalence of thyroid disease in rapid cycling than in non-rapid cycling patients. The high prevalence of thyroid disease in our patients may simply reflect the fact that most of the patients were women, that thyroid disease is much more common in women than in men, and that most of the patients had been treated with lithium, a known thyrotoxin.

Ninety-two percent of the fifty-one rapid cycling patients were women, a finding that is consistent with trends observed in other studies. Although nearly all the rapid cycling patients were women, we found no convincing evidence that the rapid cycles were generated by the menstrual cycle.

By history, treatment with antidepressant drugs was associated with reversible rapid cycling in approximately 50% of the rapid cycling patients; patients experienced slowing or cessation of cycling when the drugs were withdrawn. Prospective, longitudinal observations of 30% of the patients confirmed the association between antidepressants and rapid cycling.

Withdrawal of antidepressants seemed to be a useful approach to the treatment of a significant number of rapid cycling patients. Fourteen percent of the fifty-one patients eventually stabilized after withdrawal of antidepressants while they continued to be treated with lithium carbonate alone. In some patients who ultimately responded to treatment with lithium alone, lithium had been

ineffective in treating antidepressant-induced rapid cycling.

The results of this study provide some insights into the causes of frequent recurrences of affective illness, and can be used to improve the treatment of rapid cycling patients. First, the fact that women are much more susceptible to rapid cycling forms of affective illness is an important clue to its causation. Although the incidence of thyroid disease is no more common in rapid cycling patients than in other groups of women who have been exposed to lithium, women's vulnerability to thyroid disease might nevertheless be related to their vulnerability to rapid cycling. The observation that rapid mood cycles were not synchronized with menstrual cycles suggests that the manic-depressive cycles are not driven by the menstrual cycle and therefore that treatment strategies aimed at suppression of the menstrual cycle would not necessarily be likely to suppress the manic-depressive cycles. The observation that antidepressants may have been responsible for rapid cycling in half the cases has obvious implications for the prevention and treatment of rapid cycling, and it provides a clue to possible neurochemical causes of rapid cycling.

Seasonal Affective Disorder With Recurrent *Summer* Depression
Thomas A. Wehr, M.D.

Affective illness is inherently recurrent, and there is considerable evidence that patterns of recurrence in affective illness are strongly influenced by seasonal factors. Specifically, episodes of depression are more likely to begin in the spring and in the fall than at other times of year. Sometimes episodes recur on an annual basis producing regular patterns of summer (spring onset) or winter (fall onset) depressions, conditions we have termed Seasonal Affective Disorders (SAD). Seasonal influences are important because they suggest that depression might be caused by changes in the physical environment and that depression might be treated by manipulations of the physical environment. For a number of years we have investigated depressions that regularly recur in the winter, and there is now substantial evidence that these depressions may be caused by deficiency in light and that they can be treated by augmentation of light exposure.

About two years ago we identified a population of patients who regularly became depressed in the summer. We have investigated clinical features of summer depression, and we have tried to identify possible environmental causes of summer depression by assessing patients' responses to experimental manipulations of environmental temperature and light during their depressed state and during their well state.

As part of our investigation of the clinical features of seasonally recurrent depression we were able to compare the symptoms and diagnoses of patients with summer depression with those of patients with winter depression. Patients in both groups were assessed with structured interviews and were prospectively observed to become depressed during the season when they usually became depressed. We found that patients with winter depression were more likely to have so-called atypical features of depression, increased appetite, carbohydrate craving, weight gain, increased sleep, and psychomotor retardation. Patients with summer depression were more likely to have endogenous features of depression, decreased appetite, weight loss, decreased sleep, and psychomotor agitation. Twenty-five percent of patients with summer depression also had co-existing lifetime diagnoses of anxiety disorders (panic disorder, agoraphobia, social phobia), whereas none of the patients with winter depression had anxiety diagnoses. About half of the patients in each group had a bipolar form of affective illness (mostly bipolar II with hypomania) and half had a unipolar form. We also studied some biological correlates of seasonal mood changes in the summer depressives. Thyroid axis hormone levels (TSH, T3, T4, and Free T4) showed statistically significant seasonal variations with lowest values in the summer and highest values in the winter.

The association of endogenous symptoms with summer depression and atypical symptoms with winter depression raises the possibility that these two contrasting types of depression may be caused by exaggerated responses of physiological mechanisms that normally facilitate the organism's adaptations to conditions that prevail in the summer and winter, respectively.

Patients participated in two experiments designed to evaluate the possible role of seasonal changes in environmental temperature and light as causes of their summer depressions. In the summer, when patients became depressed, they were exposed to two treatment conditions: 1) isolation from bright light (with special neutral density glasses) and exposure to darkness, and 2) isolation from heat (with airconditioning) and exposure to cold (40° F). We found that patients significantly improved after both types of treatment with approximately 50% reductions in HRSD

scores. There are several possible explanations for this result: 1) both temperature and light are capable of regulating mood; 2) the effects of temperature and light were confounded in the study; 3) some other factor(s) that were not controlled in the study (e.g., hospitalization) were responsible for the improvements.

In the late winter and early spring, when patients were well, they were exposed to two experimental conditions: 1) exposure to bright light and 2) exposure to heat and humidity. As assessed by the HRSD, the patients' clinical state worsened during the hot, humid condition but not during the bright light condition. Hamilton depression ratings during the hot, humid condition approached values that were seen during spontaneous relapses in the summer, and differences between the heat and light conditions were statistically significant.

Taken together, these results suggest that increased heat and humidity in the summer may trigger summer depressions, and that manipulations of temperature and humidity might be used to treat or prevent this condition. These possibilities require further investigation.

Overview of the Winter Seasonality Study and the Therapeutic Effects of Light, 1987 - 1988

Norman E. Rosenthal, M.D.

In previous years we have described the syndrome of seasonal affective disorder (SAD), a condition characterized by recurrent fall-winter depressions alternating with non-depressed periods in the spring and summer. In the four years since our original description, a version of the syndrome has been accepted into the standardized manual for psychiatric diagnoses, the DSM-III-R. We have shown that treatment of SAD patients with bright artificial light, entering via the eyes, is capable of reversing the winter depressive symptoms of SAD. The efficacy of phototherapy in SAD has been replicated by several other groups and it is by now regarded as a standard treatment for this condition.

This past year we have extended our studies of SAD and phototherapy in four different directions: 1. An exploration of other groups who might benefit from phototherapy, and the development of a portable device for administering light therapy; 2. An examination of the possible biological abnormalities underlying SAD and the biological effects of light; and 3. Epidemiological investigations of seasonal changes in the population. These developments are discussed in three separate reports. In a fourth report we describe and discuss our use of bright light in the treatment of a different condition, delayed sleep phase syndrome, where we have attempted to normalize the timing of sleeping and waking by properly timed exposures to light and dark.

1. Effects of Light Interventions on Mood and Behavior:

Since we first demonstrated that the winter symptoms of SAD could be successfully treated with bright artificial light, we have questioned whether this finding could be generalized to broader segments of the general population. This past year we targeted two such populations: a representative sample of the general population of Montgomery County; and a normal elderly population, living in local residential housing complexes. The latter group appeared to be particularly promising candidates for phototherapy, given the decline in visual ability and deprivation of natural light in the winter, to which the elderly are susceptible. In fact, the elderly population did not report any improvement following exposure to bright environmental light, which actually made them feel worse. The representative sample of the general population also failed to derive any benefit as a group from phototherpy, though a subgroup of individuals, particularly prone to winter depressive changes appeared to benefit.

These findings corroborated our earlier impressions that, despite the conspicuous success of enhanced environmental lighting in individuals with regular winter mood and behavior difficulties, such interventions should not be regarded as generally beneficial for the population at large, lighting levels that may be therapeutic for some may actually be toxic for others.

For the past eight years we have treated patients by means of large, cumbersome metal boxes, containing 6 fluorescent tubes, a structure that was necessary for generating therapeutic levels of light for patients sitting at a distance of three feet from the fixture. This set-up was inconvenient, restrictive and created problems of compliance for patients in many situations. The time was clearly ripe for a more convenient and portable light source. This past year we constructed such a light source, a welder's helmet with a 4-watt fluorescent bulb attached to its front. Given the proximity of the light source to the wearer's eyes, it is possible to deliver therapeutic levels of light to the eyes, despite the low wattage of the bulb. Six patients with SAD were treated for one week with the light fixture and responded very well to it. After withdrawal for one week subjects relapsed. The patients reported that the fixture was both comfortable and free of undesirable

effects, and some were reluctant to give it back at the end of the study. This highly promising beginning suggests that it would be worthwhile to pursue the development of this fixture in future studies.

2. An examination of the possible biological abnormalities underlying SAD and the biological effects of light:

A. The Psychobiology of SAD and Biological Effects of Light:

Since the initial description of SAD in 1984 a few biological markers of the condition have been noted. The oversleeping reported by patients during their winter depressions has been confirmed by EEG and there has been a tendency for delta sleep to decrease in the winter in patients as compared with normal controls. In the past we found an increase in plasma T4 in SAD patients compared to controls. We have also found abnormal hormonal and psychological responses to the serotonin agonist, m-CPP, suggesting an abnormality in serotonergic transmission in patients with SAD.

We have found numerous biological changes following treatment of SAD patients with bright light in both morning and evening. These include: 1. enhancement of the P300 component of the event-related potential in response to visual stimulation; 2. Normalization of peripheral blood lymphocyte response to mitogen stimulation, which appears to be exaggerated at baseline in SAD patients; 3. Increased plasma norepinephrine levels, the degree of increase being directly proportional to the antidepressant effects of light; 4. Normalization of the psychological, though not the hormonal, responses to m-CPP.

Lewy has reported that patients with SAD show abnormally delayed circadian rhythms, as determined by measuring melatonin onset times in patients while they are in dim lighting conditions. He has postulated that the fundamental abnormality in SAD in abnormally timed circadian rhythms, which are normalized by appropriately timed light treatments. Thus, he has shown that light treatment in the morning is supeior in most SAD patients to an equivalent amount of evening light treatment. A second circadian theory of SAD and the mechanism of phototherapy has been advanced by Czeisler, who has suggested that the fundamental abnormality is one of amplitude, which is excessively flat in patients and which is enhanced by light treatment. This past year we have continued to use our earlier paradigm, comparing SAD patients on stable light treatment regimens (2.5 hours of bright light in the morning plus 2.5 hours in the evening) with untreated patients and normal controls. We have concentrated on circadian patterns of several plasma hormones of interest, as well as on core body temperature. The results of these studies enable us to address the above two circadian theories.

We found abnormally low overnight plasma secretion of melatonin, prolactin, TSH and growth hormone, but no abnormality in cortisol secretion. There was no abnormality in circadian phase for any of the plasma hormones. Successful light treatment did not alter the phase or amplitude of any of the plasma hormones significantly. Circadian profiles of core body temperature were no different in normals and untreated SAD patients. However, phototherapy was accompanied by a significant lowering of overnight core body temperature. As with the plasma hormones, the temperature data revealed no discernible abnormality of circadian phase, nor did effective light therapy result in any detectable significant shift of circadian phase.

B. The administration of metergoline to SAD patients receiving phototherapy:

Last year we showed that patients with SAD have abnormal responses to the serotonin agonist, m-CPP. We followed up this study with a trial of the serotonin antagoinst, metergoline, administered to patients who were on stable phototherapy. If modification of serotonergic transmission is important in mediating the response of SAD patients to phototherapy, then metergoline might modify this response.

Eight SAD patients who were being treated with phototherapy were given oral metergoline and placebo in a double-blind crossover study. There was no difference in mood response to drug and placebo, a finding that does not provide further support for the importance of serotonergic mechanisms in phototherapy.

3. The Prevalence of Seasonal Changes in the General Population:

Although problematic seasonal changes appear to be widespread, to judge by the ease with which researchers at different centers have been able to recruit large numbers of research subjects, no systematic population studies of such seasonal changes have been published to date. We have set out to document the prevalence of such seasonal changes in three different studies: 1. A telephone survey in Montgomery County, Maryland; 2. A mail-out survey at four different sites, at four different latitudes; and 3. A questionnaire study in doctors' offices at three of the four latitudes used in study #2. In all three studies we used versions of the Seasonal Pattern Assessment Questionnaire (SPAQ) developed by our group.

In the Montgomery County Telephone Survey, 416 subjects were selected and interviewed by a method of random digit dialing. Ninety-two percent of all subjects called completed the interview. Of these 92% reported varying degrees of seasonal changes in mood and behavior, and 27% reported that these changes were a problem. We developed criteria for SAD, subsyndromal SAD and summer SAD, based on SPAQ criteria and, on the basis of these criteria, we estimated the prevalence of these conditions in Montgomery County at 4.3%, 13.5% and 0.7% respectively. We interviewed a subgroup of 40 of the 416 subjects and, based on these clinical interviews, found the above estimates to be rather conservative. Those individuals who dislike winter were found to outnumber those disliking the summer by about 9 to 2. The predominant population trend was towards increased eating, increased sleeping and weight gain in the winter, changes that are in the same direction as those found in SAD.

In the four latitude mailout study, SPAQs were sent to 1000 residents at three sites: Nashua, NH (42.5°N); Montgomery Co., MD (39°N); and Sarasota, FL (27°N). An attempt was made to control for sex and only subjects over 16 were included. The methodology was based on a mail-out survey in which 400 SPAQs were sent out in New York City (40°N) the previous winter (Terman, 1987), the results of which were included for comparison. Results showed a strong correlation between latitude and a combination of SAD and S-SAD (r=1.0); however, we found no such correlation for summer SAD. A regression line based on latitudinal distribution of SAD and S-SAD at the four centers was used to derive estimates of the prevalence rates of these conditions within the continental U.S. Using this method, we estimated the prevalence of these two conditions at approximately 10.8 million cases of SAD and 25.3 million cases of S-SAD were estimated.

In the third study we examined the relationship between latitude and seasonality in non-psychiatric doctors' office patients in Nashua, Washington, D.C. and Sarasota in order to compare the prevalence among patients and the non-patients of the study outlined above. Although there was no correlation between latitude and winter SAD for medical patients, there was a strong correlation between latitude and both S-SAD and summer SAD for this group.

These three studies have helped to define the scope of the problem which seasonality presents to the general population. Since these problems affect approximately one quarter of the population to some degree and since they may be treatable by modication of the physical environment, seasonal affective disorders should be of interest to public health workers. Further, more systematic studies of the prevalence of seasonal problems are clearly warranted.

4. Treatment of Delayed Sleep Phase Syndrome with Light:

Delayed sleep phase syndrome (DSPS) is characterized by a chronic inability to fall asleep in the evening and to wake up refreshed early in the morning. These patients have an abnormally delayed sleep schedule in spite of normal sleep architecture. Traditionally they have been treated with chronotherapy, by which process they are asked to go to sleep a few hours later each night until the desired sleep onset time is reached. Although successful in some cases, this treatment is inconvenient and its results are frequentlyb short-lived. There is mounting evidence that the the light-dark cycle is capable of entraining human circadian rhythms, especially when bright light is used. The goal of this study is to investigate the possibility of treating DSPS by manipulating the light-dark cycle.

Subjects were recruited via the media. Screening questionnaires were sent to all individuals and suitable candidates were invited for screeing interviews. Patients were included in the protocol on the basis of such clinical screening and polysomnography. Thus far we have evaluated the screening questionnaires of 178 people who met our criteria for DSPS and have evaluated the clinical and demographic features of this population.

We have treated patients in a crossover study with two light-dark regimens: 1) 2 weeks of daily exposures to bright (full spectrum, 2500 lux) light from 7:00 a.m. to 9:00 a.m. and dark goggles from 4:00 p.m. to nightfall. and 2) 2 weeks of dim light (300 lux) from 7:00 a.m. to 9:00 a.m. and clear goggles from 4:00 p.m. to nightfall.

Of nine patients treated under both conditions, the bright light-dark goggle condition has proven superior to the control treatment, as measured by self-report and morning sleep latency studies. Several of these patients have chosen to continue bright light treatment after the conclusion of the formal protocol. These findings suggest that judiciously timed bright and dark exposures are of therapeutic value in DSPS, where they effectively advance patients' circadian rhythms.

The successful phase-shifting of patients with DSPS offers hope to others whose circadian rhythms are disturbed, such as shift-workers or those suffering jet lag. It would be of interest to evaluate the effects of appropriately timed exposures to bright light and dark in these indiduals.

Psychoneuroimmunology Program
Larry Tamarkin, Ph.D.

The role of the brain in mediating good health is now being appreciated. Psychoneuroimmunology is an area of research which focuses on the basic mechanisms by which the brain and the body interact to affect good or poor health. Specifically, psychoneuroimmunology combines the disciplines of neuroscience, endocrinology, and immunology into an integrated approach to investigate mind-body responses to environmental and systemic challenges. The laboratory has shown that physiologic stress can directly affect the immune system. Acute stress was shown to be immunosuppressive (as determined by an in vitro measure of the cellular immune response), while chronic physiologic stress appeared to be immunoenhancing (as determined by the same in vitro test). This study, conducted in animals, indicates that chronic and acute stress are handled differently by the neuroendocrine-immune system complex. Research is being conducted to determine the mechanism by which the immune response is first suppressed and then rebounds. In vitro data suggest that acute exposure to a glucocorticoid (a stress hormone secreted from the adrenal gland) is profoundly suppressive to the cellular immune response, while the effect of chronic exposure to this hormone is not clear. The impact of physiologic and environmental challenges on the internal milieu are being assessed to more clearly depict the endocrine events leading to the observed changes in the cellular immune response.

The primary focus of the laboratory's research has been on molecules secreted by cells of the immune system. The working hypothesis is that these molecules have effects outside the immune system. Conversely, a corollary of this hypothesis is that neuroendocrine hormones affect the function of immune cells. Essentially, the laboratory's research is targeted at understanding the bidirectional communication between the central nervous system and the immune system. This communication presumably can be accomplished through direct neural input or may be mediated via soluble factors secreted into the peripheral circulation.

One area of investigation has been to determine the effect of the interleukins on non-immune cells. Interleukins are one of the soluble proteins secreted by immune cells. Traditionally, their function has been viewed as local growth factors that stimulate the clonal (single cell) growth of specific immune cells. The immune cells that are stimulated to secrete interleukins are cells in the general circulation. Thus, it is possible that these molecules could be carried in the blood stream to act on other cells in the body. This has been our working hypothesis, which has been tested in vitro and in vivo on hormone dependent human breast cancer cells.

Breast cancer cells were chosen as a model system to test the direct effect of interleukin 1 (IL-1; a secretory product of activated macrophages) and interleukin 2 (IL-2; a secretory product of activated T-lymphocytes). We have found that interleukin 1 inhibits the growth of hormone-dependent breast cancer cells, while having no effect on hormone-independent breast cancer cells. In other words, IL-1 affects cells that are known to be responsive to estrogen, but has no effect on cells which are unresponsive to estrogen. The interaction between estrogen and IL-1 sensitivity is an area of current research.

Mechanistically, we have identified an IL-1 receptor on the hormone-dependent breast cancer cells, which was not present on the hormone-independent breast cancer cells. The affinity and number of sites per cell were similar to that found on T-lymphocytes (known target cells for IL-1). Additionally, by cross-linking experiments we have found that the IL-1 receptor has a molecular weight of approximately 80,000, which is similar to that shown for the T-lymphocyte IL-1 receptor.

To understand how IL-1 affects these cells, cell cycle growth analyses were performed. This line of investigation required the use of a state-of-the-art flow cytometer to analyze the percentage of the cells in the various stages of the cell cycle. This instrument

is capable of identifying the number of nuclei in the early stage of the cell cycle (G_0/G_1), the synthetic stage of the cycle (S), and the final (mitotic) stage of the cycle (G_2/M), giving us a complete profile of the effect of IL-1 on the growth of these cells. The data clearly show that IL-1 arrests the cells in the first stage (G_0/G_1) of the cell cycle, and again the effect is specific to hormone dependent breast cancer cells. Subsequent studies have also shown that this cell growth inhibition is accompanied by the induction of a specific 27,000 molecular weight protein. These data clearly indicate that IL-1 affects specific functions of these cells and argues that this molecule can act as an endocrine hormone. Future studies are planned to expand this investigation to pituitary cells and other estrogen responsive target tissues, including the central nervous system.

The effect of IL-2 on these cells is less clear. In vitro IL-2 does induce an inhibitory effect on growth, but the response is very dose dependent. In vivo IL-2 reduced the tumor burden of "nude" mice implanted with hormone-dependent human breast cancer cells, but had no effect on hormone-independent human breast cancer cells (note: "nude" mice have no thymus and lack a competent cellular immune system). These data suggest that exogenously admininstered IL-2, in the absence of any T-lymphocytes is capable of directly reducing a hormone-dependent tumor. Future studies are focused on understanding the mechanism of this response and perhaps understanding the role of IL-2 in the etiology of tumor surveillance and immunocompetence.

This interest in understanding immunocompetence has led us to investigate whether IL-2 levels can be detected in the peripheral circulation. To accomplish this an enzyme-linked immunosorbent assay (ELISA) assay has been developed, which has a limit of sensitivity of 100 attomoles (10^{-18} g/L) and can detect IL-2 in human plasma. We are also characterizing the distribution of the cells of the immune system by flow cytometry. The goal of this project is to develop a battery of tests that will give us a "snap shot" of the functional state of the immune system.

A series of research projects were initiated to determine if there are molecules common to the immune and endocrine systems. Specifically, we investigated if the precursor to adrenocorticotropin (ACTH) and ß-endorphin, pro-opiomelanocortin (POMC), exists in lymphocytes or macrophages (primary or cell lines). Immunologic data have been presented by other laboratories that indicate that lymphocytes and macrophage have the capacity to secrete ACTH in response to systemic challenges. Our data do not support these conclusions. We could not detect the presence of the messenger RNA for POMC in lymphocytes.

Our hypothesis was that molecules from the endocrine system could affect cells of the immune system. Our investigations have led us to examine the role of ACTH on peritoneal macrophages and on a number of human and mouse macrophage cell lines. These studies indicate that ACTH itself can stimulate the growth of some of these cell lines. We also have been investigating the ability of ACTH to stimulate the production of two macrophage secretory products: IL-1 and tumor necrosis factor (TNF). This investigation required the development of quantitative assays for the secretion of IL-1 and TNF and the development of Northern blot analysis for mRNA expression of both macrophage products. The data indicate that ACTH in concert with lipopolysaccharide (LPS) can cause the secretion of TNF. [note: TNF has been shown to have effects outside the immune system and in particular may act on the temperature center of the hypothalamus to regulate fever.] The mRNA analysis is ongoing.

In summary the program has identified biochemical connections between the neuroendocrine and immune systems. The continued focus of this research is on basic mechanisms of this communication and the long range goal is to assess behavioral strategies that can be positively transduced to the neuroendocrine-immune system leading to good health.

ANTIDEPRESSANT TREATMENTS AND THE MAMMALIAN CIRCADIAN SYSTEM

Wallace C. Duncan, Jr.

Introduction

Biological rhythms are disturbed in the majority of patients with affective disorder. These abnormal rhythms include disruptions of circannual or seasonal rhythms, as observed in seasonal affective disorder, disruptions of circadian or daily rhythms as observed in hyposomnia or hypersomnia, and disruptions of ultradian rhythms such as the disturbed REM cycle that frequently accompanies primary depression. This project focuses on the effects of antidepressant drugs and light on the mammalian circadian system. We have utilized the Syrian hamster as an animal model. The general hypothesis under investigation is that the mechanism of the antidepressant response to chronic drug therapy includes effects on the mammalian circadian system. Our results support this hypothesis.

The mammalian circadian system is a precisely organized network of physiological processes. Circadian studies focus not only on the spatial (neuroanatomical) aspects of this organization, but also on important temporal aspects of this organization. There are two arguments for combining the spatial and temporal domains within a single investigation. Especially in behavioral studies it is not sufficient to answer the questions "where" or "how" these elements are distributed to form a static system. It is also critical to answer "when" these elements integrate to yield a dynamic and functional system. Second, in combination, these two dimensions.provide additional analytical capacity not otherwise available. We utilize this approach to gain insights regarding the functional aspects of basic elements which constitute the mammalian circadian system. Second, we can exploit the temporal and spatial domains to dissect the mechanism of antidepressant treatments. This is important since our previous work suggests that the timing of antidepressant treatment is critical for maximizing clinical benefits.

Our studies have four areas of interest. The first is to understand the effect of antidepressant drugs on the endogenous expression of the mammalian circadian clock or "pacemaker" that ultimately controls the daily rhythm of neurochemicals, physiological processes and behavior. Second, we are interested in the capacity of these drugs to alter the responsiveness of the circadian pacemaker to environmental factors. The most important of these is probably light. Third we are interested in the effect of antidepressant drugs on secondary processes such as the temporal pattern of metabolic rate and body temperature. Finally we want to understand more about the effects of light on regulating the levels of neurochemicals which control the expression of the circadian pacemaker. The following discussions review our progress in these areas.

Antidepressant Pharmacology of the Mammalian Circadian Pacemaker

Clorgyline, a monoamine oxidase inhibitor with antidepressant properties, alters the endogenous expression of the circadian system by a) delaying a large portion of motor activity to the second half of the activity phase, b) reducing the duration of the rest phase by about 30% and c) decreasing the the frequency of the daily biological clock. In these past studies, we utilized wheel-running as an index of circadian pacemaker expression. During the past year we have extended our studies to include clorgyline's effects on EEG sleep, telemetred body temperature and telemetred gross motility. We have observed recently that clorgyline's effects on delaying a large portion of wheel-running activity and reducing the duration of rest is not dependent on running-wheel availability. Similar effects are observed in EEG documented wakefulness and locomotor motility scores. This extended

observations suggest that clorgyline's effects on the activity-rest cycle are due to the altered expression of the primary circadian pacemaker. The effect on the primary pacemaker is sufficient to explain the altered expression of a variety of behaviors such as EEG wakefulness, wheel-running and locomotor motility.

We have extensively explored the effect of clorgyline on the responsiveness of the circadian system to continuous environmental light. These studies indicate that during chronic clorgyline treatment a) the activity-rest cyle becomes progressively more disorganized as the intensity of continuous light is increased. Behaviorally, this phenemenon is similar to eliminating the site of the circadian pacemaker. Although it is possible that the combination of bright light and drug treatment disorganizes the circadian pacemaker, it is also possible that the pacemaker remains functionally intact and the disruption of behavior occurs downstream from pacemaker control. There are techniques for dissecting these possibilities which we are currently pursueing.

Metabolic Studies

Antidepressant drugs are often observed to produce changes in the body mass of patients receiving treatment. We have previously observed that chronic treatment with the monoamine oxidase inhibitor clorgyline alters the body mass of Syrian hamsters. Hamsters treated with this drug failed to increase body mass as did control hamsters. The change in body mass was primarily due to an effect on body lipid content. In our earlier studies we also observed that the failure to increase body mass was not due to a decrease in caloric input since clorgyline-treated hamsters exhibited greater food intake than saline-treated hamsters. Thus, clorgyline produced a condition of negative energy balance compared to control hamsters.

We have designed a chamber that will allow online, simultaneous measurement of oxygen consumption, motor activity, food intake and body temperature to more fully examine the interaction of environment, behavior and metabolism in antidepressant drug-treated hamsters, . Ambient temperature can be controlled between 5 and 40 degrees centigrade. Ambient lighting can be progammed to follow numerous regimens including seasonal fluctuations in daylength. The relationship between parameters which determine energy balance, i.e., energy lost as heat (either during motor activity or during thermoregulation), or energy gained during food intake, can be explored within a circadian perspective.

We have recently observed a significant decrease in the core body temperature of clorgyline treated hamsters. This effect appears to be uniformly distributed throughout the circadian cycle and is approximatly one-half degree centigrade. This observation is consistent with our interpretation that clorgyline induces a negative energy balance in Syrian hamsters. We are currently evaluating potential drug effects on brain temperature.

Antidepressant Drugs and Circadian Pacemaker Sensitivity to Light

One of the central features of the mammalian circadian pacemaker is to entrain a variety of physiological and behavioral processes to the daily light dark cycle. The circadian pacemaker has the capacity to advance or delay its phase in order to maintain proper timing with the geophysical day-night cycle. The phase-advance and phase-delay mechanisms exhibit independent profiles of responsiveness to the light-dark cycle. Phase-advances show maximum responsiveness to light at dawn and phase-delays show maximum responsiveness to light at dusk.

Phase disorders of the sleep-wake cycle such as phase-delay sleep syndrome, or sleep disturbances that accompany depression prior to, or during drug treatment may be caused by the inability of the circadian pacemaker to properly advance or delay its phase in relationship to the external light-dark cycle. Thus, these disorders may be considered in one sense, dysfunctions of the entrainment mechanism. Antidepressant drug treatments may alter the sensitivity of the circadian pacemaker to light and thus establish non-pathological phase relationships between the pacemaker and the environment. We have been exploring the effect of the monoamine oxidase inhibitor clorgyline on the sensitivity of the circadian pacemaker to light.

We have previously observed that the monoamine oxidase inhibitor, clorgyline, alters the responsiveness of the circadian pacemaker to light. However, the effect of clorgyline on threshold or saturation response was not been described. We have determined that chronic treatment with the monoamine oxidase inhibitor clorgyline increases the response threshold of the circadian pacemaker to light. Thus, these data are consistent with the hypothesis that antidepressant drugs alter the sensitivity of the circadian pacemaker to light. Further research will be required to determine if other classes of antidepressant drugs such as lithium or tricyclic antidepressants possess similar or unique characteristics in their capacity to alter the sensitivity of the circadian pacemaker to light.

The Effects of Light on NPY mRNA Synthesis

Recent studies have identified a circadian pacemaker located within the suprachiasmatic nucleus (SCN) as a central component that regulates the rhythmic expression of a variety of mammalian circadian processes. The SCN performs three important functions. It entrains circadian rhythms to the daily light-dark cycle. It generates an endogenous circadian rhythm of approximately twenty-four hours which then controls the period of secondary processes. Finally, it integrates these rhythms into a single, functional circadian system with coordinated temporal realtionships between multiple circadian rhythms. Many of these functions may be compromised in affective disorder.

There are two major visual inputs to the SCN of the hypothalamus. The first is a direct projection from the retina, the retinohypothalamic tract (RHT). The neurotransmitter of this projection has not been clearly identified, but its function is to facilitate entrainment to the environmental light-dark cycle. The second is an indirect visual projection from the intergeniculate leaflet (IGL) cells of the lateral geniculate nucleus (GHT) to the SCN. This projection utilizes neuropeptide Y (NPY) and appears to convey information specifically pertaining to the level of ambient illumination to the SCN. Since a) the GHT is one of only three major projections to the SCN, b) NPY is contained within the GHT and is the only transmitter identified that relays visual information to the SCN, c) the functional significance of the GHT and NPY is closely related to the circadian system, and d) decreased levels of NPY have been observed in affective disorder, we have begun to investigate the circadian and environmental regulation of NPY mRNA synthesis within the GHT

Using in situ hybridization histochemical techniques, other investigators have recently demonstrated the presence of mRNA encoding for NPY in rat arcuate nuclei and cortex. Using in situ hybridization, we have identified NPY mRNA in the vicinity of the IGL cells. These results indicate that in situ hybridization may be a viable technique for further exploring a) the circadian regulation of NPY mRNA synthesis and b) the effect of different ambient light levels on the control of NPY mRNA. These experiments are currently in progress.

Summary

During the past year we have made rapid and significant progress in understanding the effects of antidepressant treatments on the mammalian circadian system. Behavioral circadian studies have been extended to include continuously monitored EEG sleep and wakefulness, wheel-running, and locomotor motility. Physiological studies include continuously monitored body and brain temperature , oxygen consumption and heat production. Compelling evidence indicates that chronic antidepressant drug treatment alters the sensitivity of the circadian pacemaker to light. This result suggests a possible relationship between the reported hypersensitivity to light in bipolar patients and a previously undescribed mechanism of antidepressant drug treatment. Further evidence indicates drug treatment increases morning activity possibly through effects on the circadian pacemaker. These results suggest a possible common mechanism linking the drug-induced antidepressant response with the antidepressant response to sleep deprivation which is maximum during the early morning. We have observed that chronic treatment with the MAOI clorgyline produces a significant hypothermia and we are currently investigating other metabolic effects of chronic drug treatment. Finally, we are opening a new avenue of investigation utilizing in situ hybidization techniques to explore the effects of envvironmental factors such as light on regulation of neuropeptides that have been implicated in the control of the mammalian circadian system and depression.

Physiology of Sleep and Sleep Loss
C. A. Everson, Ph.D.

This is a prospective overview of new avenues of basic sleep research to be conducted in the animal laboratories of the Clinical Psychobiology Branch.

The Clinical Psychobiology Branch is strongly committed to sleep research, as it has been for many years. The study of sleep falls solidly into the realm of psychobiological research: sleep is defined first and foremost as a stereotypic behavior of immobility and quiescence which is associated with a unique constellation of physiological events.

The study of sleep physiology is important because the function(s) of sleep remain unknown, even though sleep as a phenomenon has been well described. Several features of sleep indicate that it must serve an important biological function. For example, sleep is ubiquitous among mammals, birds, and reptiles. Rest does not substitute for sleep. Sleep rebounds in duration and intensity after sleep loss, which suggests compensation for a postponed biological need. And lastly, sleep deprived animals can withstand sleep loss only a few days longer than food deprived rats can survive starvation, indicating that sleep is physiologically vital. In effect, the function of sleep may be one of the great unsolved biological mysteries of our time.

The long-term goal of our animal research is to determine what is accomplished during sleep which cannot be accomplished during wakefulness or rest. Outcomes of this research will provide basic biological knowledge. Furthermore, the more we know about processes which underlie sleep, the sooner we will be able to determine a role for sleep in clinical pathologies. It is possible that subtle changes in sleep are involved in eticlogies of disease processes. If this can be determined, manipulation of sleep may aid recovery from disease. Companion reports from the Clinical Psychobiology Branch, by Dr. Thomas Wehr and colleagues, point to the possible implications of sleep dysfunction in psychiatric disorders, such as depression. Some depressed patients derive immediate benefit from sleep deprivation. Outcomes of animal experiments on the physiology of sleep and sleep loss may help elucidate the role of sleep in psychopathology.

Our main approach is to investigate physiological changes which underlie the major pathological effects of prolonged sleep loss. Chronic sleep loss in rats caused the following main effects: a progressive increase in energy expenditure to a level doubled that of baseline; skin and blood pathologies which may be attributable to problems in protein biosynthetic pathways; and an eventual inability to maintain body temperature in spite of high energy expenditure. Taken together, the sleep loss effects comprise a syndrome unlike any clinical disorder in the biomedical literature. However, each of these effects may reflect more than one impaired biological process. Our task is to find the underlying processes on which the symptoms may be dependent. By the nature of the main regulatory deficits, we may then be able to go one step further and infer a function normally fulfilled by sleep. Also, symptoms of short-term sleep deprivation in animals and humans, which are generally unremarkable and ambiguous, may acquire meaning as we map out regulatory adjustments during extended deprivation.

In other experiments, sleep will be permitted ad lib and used as a bioassay to investigate interrelationships between sleep and other regulatory systems. For example, metabolic rate and nutritional status will be alterred to determine effects on sleep time. If sleep time is reliably influenced by the manipulations, we can then evaluate the underlying process(es) which may be exerting control over sleep.

We plan to pursue a second objective concurrent to undertaking the four experiments summarized later in this report. Because sleep and sleep loss represent relatively uncharted states of regulation for many systems, such as endocrine and neurochemical systems, we plan to collaborate with other scientists in these specialized areas. A joint approach to certain issues can help fulfill two research agendas: 1) investigation of the behavior of a particular mechanism or system during sleep, and 2) delineation of the importance of that mechanism in sleep or sleep loss effects. Momentum behind our collaborative relationships is growing. To date, potential collaborative endeavors include 1) investigation of the metabolic activity of various brain regions during sleep loss and recovery sleep, 2) evaluation of the activity of peripheral immune defense

mechanisms during sleep loss, and 3) searching for mechanisms underlying a dramatic decrease in plasma levels of thyroxine and triiodothyronine in the sleep deprived rat. We will undertake collaborative investigations as we get the initial experiments underway and increase our technical capacity via laboratory space and support staff.

Below is a summary of four initial experiments:

Physiology of Sleep Loss

The aim of this study is to determine which sleep loss symptoms in the rat indicate a physiological system primarily affected by deprivation, and which symptoms are secondary effects.

The first step in studying sleep loss in rats was to profile biochemical and physiological changes after long-term deprivation. Everson and colleagues at the University of Chicago Sleep Research Laboratory found that chronic sleep loss resulted in a specific syndrome, rather than a generalized impairment of all physiological systems. Sleep loss symptoms can be grouped under five main deprivation effects:

1) *Mortality*. Totally sleep deprived (TSD) rats died, or were sacrificed when death appeared imminent, after 20.9 (SD = 5.9) days of sleep loss. Yoked control (TSC) rats appeared as though they could continue living under the experimental conditions.

(Some results for TSD rats will be described in terms of quarters of survival time. Each rat's survival time was divided into quarters and data were averaged for each quarterly bin, first within rats and then across rats.)

2) *A progressive increase in food intake concomitant with body weight loss.* Daily food intake eventually reached 80% above baseline values during the last quarter of survival. Mean weight loss from baseline during the same period was 12%. This catabolic state could not be explained by malabsorption of nutrients, hyperactivity, diabetes, or dehydration. Indirect calorimetric measurements showed a near two-fold increase in energy expenditure.

3. *Protein-related deficits.* In spite of increased protein ingestion, plasma albumin declined progressively after the first quarter of survival, eventually reaching an average of 30% below baseline levels during the last quarter of survival. We could not find a cause of the hypoalbuminism: a) protein was not lost in the urine, b) rats did not suffer from malabsorption, and c) liver dysfunction was not indicated in the results of assays of liver enzymes or globulin electrophoresis.

During the experimental quarters three and four, TSD rats developed a normocytic anemia. The anemia was characterized by a decline in total red blood cell mass without significant replacement by immature cells; a profile similar to that found in protein malnutrition states. The anemia was most likely not caused by blood loss because hemorrhages were not seen on organs at necropsy, and patterns of mild urinary blood in both TSD and TSC rats did not parallel the developing anemia. Red blood cells were not excessively destroyed; plasma levels of conjugated and unconjugated bilirubin and the liver enzyme, GTT, remained stable and normal.

4. *Severe stereotypic ulcerative and hyperkeratotic skin lesions* located on the tail and plantar surfaces of the paws. Necrotizing vasculitis, trauma, and zinc deficiency were ruled out as causes of the lesions.

5. *Body temperature changes.* A mild increase (o.5 oC) in core temperature was concomitant with the raised energy expenditure during the first half of the experimental period. During the second half of the experimental period, core temperature declined below baseline levels, indicating a difficulty thermoregulating.

Histological examination of organs, including brain, of other TSD rats deprived by the same methods were negative.

Our first experimental aim is to search for the most fundamental sleep deprivation-induced pathology. We will begin by attempting to separate the effects of a protein-related deficiency from those of increased energy expenditure. If sleep serves to promote biosynthetic activities, as some researchers have proposed, a protein deficiency would be an expected

outcome of sleep loss. Physiologic attempts to off-set increased protein degradation, impaired biosynthetic processes, or to rid nonprotein calories could raise energy expenditure. On the other hand, increased energy expenditure from another cause may have produced excessive protein metabolism, resulting in a protein deficiency. By separating these two main sleep loss effects we will be able to evaluate whether one is secondary to the other.

The second aim is to determine whether a protein deficiency might explain several sleep loss symptoms, regardless of whether the protein loss was secondary to increased energy expenditure. Many symptoms of TSD rats resemble those of Kwashiorkor patients maintained on a diet low in proteins with relatively normal calories. These patients exhibit hyperkeratotic skin lesions with superficial ulceration, mild to severe edema, low hemoglobin, suppressed circulating lymphocytes, hypoalbuminism, normal total plasma proteins, and hair changes, symptoms which were also observed in TSD rats.

The Interaction Between Sleep Duration and Energy Expenditure

Functional relationships may exist between two processes which appear to covary, such as alterations in sleep and energy balance. Several lines of evidence suggest a strong positive relationship between metabolic rate and sleep. For example, sleep duration is highly correlated with estimates of metabolic rate across species. Young animals spend a greater proportion of their time asleep than adults. In humans, the higher the waking body temperature (which correlates positively with metabolic rate), the greater the duration of sleep. A near doubling of metabolic rate occurs in rats during long-term sleep deprivation. If sleep and energy balance are intricately related, sleep duration should be sensitive to states of increased or decreased energy utilization.

The metabolic rate of rats will be manipulated via the thyroid system to determine whether sleep amount is lawfully related to changes in metabolic status.

Sleep Effects of Essential Fatty Acid Deficiency

It has been reported that essential fatty acid deficiency (EFAD) in rats produced a near 50% increase in total sleep time. This was a rare situation in which withholding, rather than administration, of a relatively specific substance alters sleep parameters.

In this study, we will search for the mechanisms of the sleep-inducing effect in the EFAD state. Several important questions will be addressed:

1. Are there qualitative differences between sleep in normal and EFAD rats? For example, do EFAD rats sleep longer because their sleep is less intense (i.e., as measured by analysis of brain wave frequency and amplitude)?
2. Might changes in brain myelination be the cause of changes in the qualitative aspects in sleep of EFAD rats?
3. Are changes in core and brain temperature during sleep in EFAD rats similar to those seen during sleep in normal rats? Are indications of an altered energy balance reflected in the temperature measurements during sleep?
4. Which essential fatty acids are necessary to restore normal sleep? Will end products of fatty acid synthesis (e.g., prostaglandins) normalize sleep in EFAD rats?

Examination of Prostaglandin-Induced Sleep

Prostaglandins are potent, putative sleep-inducing substances. Mechanisms which underlie the effects of prostaglandins on sleep are unknown. Prostaglandins also influence core, and possibly brain, temperature. Sleep and typical patterns of sleep stage alterations are associated with changes in core and brain temperature. It is possible that prostaglandins exert their effect on sleep because they alter core and brain temperature.

This study will address mechanisms which may mediate the effects of prostaglandins on sleep, starting with an examination of the correspondence between prostaglandin-induced changes in core and brain temperature, and changes in sleep parameters.

We will proceed first with the studies on the physiology of sleep loss and the sleep effects of essential fatty acid deficiency. These two studies can potentially yield the most specific information about physiological processes involved in sleep or sleep loss. As we get more equipment and resources in place, we will conduct the studies on prostaglandin-induced sleep, the interaction between sleep duration and energy expenditure, and collaborative investigations.

ANNUAL REPORT OF THE LABORATORY OF CLINICAL SCIENCE
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1987 through September 30, 1988

Dennis L. Murphy, M.D., Chief

The Laboratory of Clinical Science (LCS) has become more basic science-oriented than clinical science-oriented with the planned move this year of one of the two clinical sections, that headed by Dr. William Potter, from the LCS to the Clinical Neuroscience Branch. Last year, the remnants of another clinical group, the Section on Biomedical Psychiatry, moved into the new Clinical Neuroendocrinology Branch headed by Dr. Philip Gold, following the departure to Harvard of the former section chief, Dr. David Jimerson. This leaves three smaller basic science sections (Analytical Biochemistry, Histopharmacology, and Comparative Studies of Brain and Behavior) and one large clinical section (Clinical Neuropharmacology) in the LCS. This has made the Laboratory more manageable. As indicated by the individual section summaries presented below, all of these groups have continued to be highly productive.

Section on Analytical Biochemistry
Sanford P. Markey, Ph.D., Chief

This Section develops and applies new analytical instrumentation to problems in neuropharmacology, especially to problems of neurotoxicity. In the area of neurotoxicity, quinolinic acid, a known convulsant and excitotoxic intermediary metabolite of dietary tryptophan, has been found to be elevated in brain extracellular fluid to potentially neurotoxic or behavior-modifying levels during infectious states. Dr. Melvyn Heyes has found higher than normal levels of quinolinic acid in the cerebrospinal fluid (CSF) of AIDS patients, in retrovirus infected animal models of AIDS, and in experimental animals receiving endotoxin. Tryptophan, labelled with the stable isotopes ^{13}C or ^{2}H, can be followed in experimental animals through the kynurenine pathway to quinolinic acid. Dr. Riccardo Boni has measured labelled quinolinic acid in the CSF of rabbits following treatment with labelled tryptophan. These techniques are being refined in order to determine quinolinic acid production and control in AIDS patients. Studies of the putative neurotoxic amino acid BMAA, a constituent of cycad plants found in areas where an ALS/PD syndrome has been endemic, have indicated that this compound is an unlikely causative agent. Dr. Mark Duncan has measured BMAA content of plant seeds and the washed flour prepared from such seeds, and has found the levels far below those required for neurotoxicity.

Idiopathic Parkinson's disease could be caused by a neurotoxin with properties like those of MPTP, a product of illicit drug preparation. Dr. Jan Johannessen has studied the metabolism, species, and age sensitivity to MPTP, and has found that the aged dog is very susceptible to low doses of MPTP, perhaps due to decreased synaptic storage capacity.

Neurochemical events in living animals are being directly measured using *in vivo* micordialysis procedures. Dr. John Hsiao has made a number of methodological advances which are consistent with computer models of the kinetic disposition of neurotransmitters in brain tissue.

Successful quantitative applications of a new mass spectrometric procedure (continuous flow-liquid secondary ion mass spectrometry) have been demonstrated by Dr. T.C.L. Wang and Ms. Shih. These new techniques will permit the direct measurement of polar, non-volatile organic compounds in biofluids.

Section on Clinical Neuropharmacology
Dennis L. Murphy, M.D., Chief

This Section explores the biochemical and behavioral pharmacology of novel as well as some standard psychoactive agents in attempts to understand both how these drugs work and, more importantly, to use these drugs to investigate normal and abnormal brain neurochemistry. Our current studies can be subdivided into three main areas of investigation: (1) a geropsychopharmacology research program focused on patients with Alzheimer's disease and elderly patients with depressive disorders; (2) psychopharmacologic studies of patients with obsessive-compulsive disorder, and (3) laboratory studies directed, in particular, towards studies of serotonin-selective agents and of opiate receptor subtypes, with special interests in processes involved in the adaptational consequences of chronic psychoactive drug administration.

Patients with Alzheimer's disease have been found to respond differentially to two postsynaptic cholinergic agonists: one agonist, arecoline, led to modest cognitive improvement but no behavioral changes; in contrast, another agonist, nicotine, elicited surprisingly marked affective changes without any cognitive alterations. Combination drug treatments are going to be increasingly explored in Alzheimer's disease using agents acting on different neurotransmitter systems (e.g., physostigmine plus L-deprenyl, cholinergic agents plus peptides like thyrotropin), as increasing evidence points to multi-system dysfunctions being involved in this disease, and as our group has clinical and laboratory evidence that thyrotropin can attenuate learning deficits produced in the anticholinergic (scopolamine) model of Alzheimer's disease.

Increasing evidence points to a serotonergic neurotransmitter subsystem abnormality in patients with obsessive-compulsive disorder (OCD). Patients who had improved during drug treatment with the serotonin selective tricyclic, clomipramine, exhibited normalization of a specific serotonergic abnormality, namely a behavioral hyperresponsivity to the serotonin agonist, m-CPP. OCD patients were also found to exhibit a brief return of symptoms when a serotonin antagonist was administered during the course of successful treatment of their disorder with clomipramine.

In the drug abuse area, rats made tolerant to morphine or naltrexone exhibited upregulation of opiate receptors via different mechanisms, which may have implications for the development of opiate dependence.

Section on Comparative Studies of Brain and Behavior
Dennis L. Murphy, M.D., Acting Chief

In the past year, the Section has continued its focus on developmental neuroscience. In a series of studies, hypothalamic lesions have been found to block the initiation of maternal behavior. The regulation of hypothalamic oxytocin receptors by estrogen and androgens is being explored. Brain oxytocin receptors have been found to be expressed in a restricted fashion in development, increasing markedly in hypothalamus at puberty. Estrogen has also been found to induce c-fos gene expression in brain.

In studies with neurotoxins, 3,4-methylelene dioxyamphetamine (MDMA) has been found to be a selective serotonin neurotoxin in non-human primate (rhesus monkey) brain. In related pharmacologic studies, MDMA administration was found to block infant separation distress in the rat.

Prenatal stress has been shown to be associated with long-term changes in brain opiate and benzodiazepine receptors. Survival of spinal cord projecting cells in the locus coeruleus was found to be increased by transection of the dorsal noradrenergic bundle.

In the next year the comparative approach will continue to be emphasized. Studies of affiliation will be extended to species on the basis of different affiliative styles (e.g., microtine voles). In addition, receptors studies and developmental neuroanatomic studies will be extended to non-human primates.

Section on Histopharmacology
David M. Jacobowitz, Ph.D., Chief

Accomplishments this year include:

The isolation and purification of the A_1 adenosine receptor from rat brain membranes. This receptor was purified about 50,000-fold to homogeneity. A single Mr 34,000 silver-stained band was observed on 1-dimensional chromatography. We plan to attempt to ascertain partial sequences of the A_1 adenosine receptor and generate an antibody to this protein.

We have isolated, purified, and raised an antibody to a novel calcium binding protein. Immunocytochemical studies reveal a remarkable localization within animal and human brains and spinal cords. This protein is prominent within all sensory neuronal inputs to the brain in addition to other interesting areas of the brain, including the substantia nigra and cerebral cortex. Proteolytic fragments of this protein reveal an 82% sequence homology with a protein (calretinin) produced by a cDNA clone in chick retina. We plan to develop a radioimmunoassay for this novel calcium binding protein and to study this protein in CSF of normal and pathological states (schizophrenia, Alzheimer's

disease, depression, multiple sclerosis). Also, quantitative studies will be carried out on animal and human brains.

Unit on Preclinical Neuropharmacology
Juan M. Saavedra, M.D., Acting Chief

It is anticipated that this Unit will be made into an independent section in recognition of the broader program function which has grown out of Dr. Saavedra's sustained efforts. He has substantially expanded his research focus to include: the pharamacologic manipulation of classes of CNS receptors possibly underlying hallucinations produced by psychotomimetics; the role of specific dopamine D_1 and D_2 receptors in the brain; the application of quantitative autoradiography to the study of receptors in human circulating cells, and the further development of techniques for determining antigens, including neuropeptides, in discrete brain areas.

Specifically, a recently available iodinated potent psychotomimetic 5-HT_2 agonist, ^{125}I-DOI, was found to bind specifically to rat cortex and claustrum. ^{125}I-LSD was shown to bind to the same areas with DOI and LSD displacing each other. Like LSD, DOI reduces 5-HT_2 binding in cortex. Taken together, these findings support a possible role of the identified binding sites in the psychotomimetic effects of these drugs.

A method with 100-fold greater sensitivity than classical radioimmuno-assay techniques has been developed which can be applied to a variety of CNS peptides which can be viewed as tissue antigens. In brief, Protein A is utilized as an immunocytochemicl reagent obviating the need for a second antibody. When coupled with autoradiography, antigens are quantifiable in single nuclei of thin brain sections. This method has been validated for such antigens of interest as tyrosine hydroxylase and angiotensin-converting enzyme.

Annual Report for the Child Psychiatry Branch
National Institute of Mental Health
October 1, 1987 - September 30, 1988

The Child Psychiatry Branch, formed in 1984, conducts research on biological aspects of child psychiatry. Neuro-radiological techniques and response to pharmacological agents are the major tools for this research. However, epidemiological and family studies are also ongoing.

Within the NIMH, there are ongoing collaborations with the LPP (Drs. Zahn and Duncan), the LCS (Drs. Murphy and Potter), and LCM (Dr. Cohen). Collaborations are also taking place with other NIH institutes: NIA (Dr. Stanley Rapoport and staff), and NIAAA (Dr. Linnoila). Collaborations with other institutions are: Dr. Agnes Whitaker (Columbia), Dr. Eric Taylor (The Maudsley Hospital) and Dr. Michael Goldstein (UCLA).

Three broad areas of clinical pathology are the focus of the Branch research. Developmental disabilities (dyslexia, autism) are being studied with Positron Emission Tomography (PET). There is a strong association between developmental disorders and many psychiatric syndromes which makes this study of general interest to mental health research. The past year has been devoted to development of appropriate auditory and visual tasks with normal controls in order to study normal reading development.

Obsessive compulsive children and adults are being followed prospectively as part of the clomipramine discontinuation study. A double-blind study of clomipramine treatment of trichotillomania (hair pulling) and onchyphagia (nail biting) is underway. Preliminary data shows that clomipramine, but not desmethylimipramine, improves these conditions. This, together with the association of OCD with basal ganglia pathology and PET data, suggest that OCD is a release of primitive grooming behaviors possibly due to pathological excitation from frontal lobe input to the striatum.

Studies of aggressive and hyperactive children occupy one-third of the branch resources. A double-blind crossover study of methylphenidate and dextroamphetamine shows that almost all of the hyperactive children respond to one or the other of these stimulants, if not to both, if a sufficiently wide dose range is tried. Preliminary analysis of CSF samples from this population has shown the importance of height correction within this age group, as well as an independent effect of puberty (Tanner staging) on CSF monoamine concentrations.

ANNUAL REPORT OF THE CLINICAL NEUROGENETICS BRANCH
National Institute of Mental Health
October 1, 1987 - September 30, 1988
Elliot S. Gershon, M. D., Chief

There has been a historic scientific paradigm shift in psychiatric genetics research, from its earlier concentration on epidemiology of diagnoses in families into its current concentration on identification of single genes which play a role in the major psychiatric disorders. The Clinical Neurogenetics Branch has played a major role in this shift. It has pioneered in genetic linkage studies in manic-depressive illness and schizophrenia. It has provided leadership to the field in the development of appropriate clinical research paradigms based on mathematical analyses and simulations, in the definition of appropriate clinical methods and pedigree configurations, and in the development of laboratory methods appropriate to separation and identification of protein and DNA polymorphisms. This branch has recently carried out some of the major initial studies in protein variation associated with psychiatric and neurologic disease, and in study of restriction fragment length polymorphisms as linkage markers in psychiatric disorders.

At this time, the Clinical Neurogenetics Branch, which was established in 1983, will divide into two branches, in recognition of the accomplishments and leadership of Dr. Carl Merril.

Section on Clinical Genetics
Elliot S. Gershon, M.D., Chief

Our investigation of inherited psychiatric disorders is an interdisciplinary effort, in gene mapping, genetic epidemiology, and pathophysiology. We have made progress this year in several of these efforts.

Current linkage-to-illness results: Chromosome 11: We initially reported a pedigree series in which no pedigrees were linked. We have since identified an Ashkenazi Jewish pedigree with bipolar illness, where current lod score with 11p15 markers HRAS1/INS is 1.1 (possible linkage). This may represent a replication of the linkage found by Egeland et al. in the Amish. Chromosome 5: A balanced translocation at 5q11 associated with schizophrenia in one family has been reported. We have identified two pedigrees with possible linkage to the glucocorticoid receptor gene (GRL) in that region. In view of these provocative but not yet statistically significant findings, we are investing considerable efforts in physical and genetic linkage mapping of the two regions, 5q11 and 11p15.

X-chromosome color blindness region (Xq28): although linkage of manic-depressive illness to this region has been reported, we have examined six new pedigrees with a molecular marker of this region (St-14). Linkage can be excluded with great certainty in this series.

Candidate genes: We have also studied genes involved in neurotransmission. The $beta_1$ and $beta_2$ adrenergic receptor genes were excluded as linkage markers to affective illness, suggesting that genetic variation in these genes is unrelated to manic-depressive illness. New genes which appear to code for a family of substrates of protein kinase C, an important enzyme in an intracellular second messenger system,

have been cloned.

Mathematical analysis: Single gene locus vulnerability in major psychiatric disorders appears to be present in some cases of bipolar disorder, and possibly in some cases of schizophrenia. We have performed mathematical analyses of the power of current methods and pedigree resources to detect linkage of markers to illness, and to identify association of particular gene variants with illness due to linkage disequilibrium, under the conditions of genetic heterogeneity and possible non-genetic causes, which may exist in manic-depressive illness and schizophrenia. Linkage can be detected with very feasible sample sizes of small families (5 or 6 affected individuals) in the presence of moderate genetic heterogeneity. Considerably larger sample sizes, however, are needed to demonstrate that heterogeneity (rather than loose linkage) is present. When flanking markers are available, both linkage and heterogeneity are more readily detectable. Within a linkage region, the causative gene mutation and alleles of neighboring genes may be associated with illness; due to linkage disequilibrium. Analysis of the power of detection of disequilibrium in the presence of moderate to great heterogeneity reveals that for several realistic parameter sets, only one or two linked pedigrees are needed.

Laboratory methodology: we have improved restriction fragment length polymorphism (RFLP) resolution on Southern blots through field inversion gel electrophoresis (FIGE). This should lead to increased detectable polymorphism for several markers of crucial interest in studies described above.

Pedigree collection for manic-depressive illness and schizophrenia molecular mapping studies continues. In manic-depressive illness, we have 15 pedigrees with more than 200 individuals in culture. In schizophrenia, we have 7 pedigrees with more than 70 individuals in culture. We also have 41 affected-sib-sets. We are continuing in an intensive effort to obtain moderate to large sized pedigrees in manic-depressive illness and schizophrenia. These collections should aid in determining the epidemiological representativeness of linkage findings. We expect that a portion of this collection will be included in the NIMH Genebank cell collection.

Susceptibility studies not attributable to single gene locus events: A longitudinal prospective study of adolescents at high risk for bipolar affective disorder is continuing. A biological marker, the suppression of plasma melatonin by light, differentiates the "high-risk" adolescents from a control adolescent group. Brain structural anatomy in familial schizophrenia was studied with magnetic resonance imaging (MRI). Compared with the control sample the schizophrenic siblings had significant volume reduction of the temporal lobes, bilaterally, and significant increase in ventricular brain ratio (VBR). These structural abnormalities may be part of the familial pathophysiology of the disease.

Section on Biochemical Genetics
Carl M. Merril, M.D., Chief

The section's major research efforts have been concentrated on the development of methods for and the study of genes and gene products in physiological and pathophysiological states. Electrophoresis and staining of proteins from cerebrospinal fluid (CSF) and brain tissue have increased detection of proteins from 300 to 1500 and 1000 to 3500 respectively.

Disease-associated proteins in the CSF have been further investigated. Purification and partial amino acid sequences have been obtained from 6 proteins: one of the proteins is identified as a transthyretin molecule, twice the molecular weight of the usual protein, and absent from the plasma. The other 5 proteins are not homologous to any of the known sequences in the NBRF protein database. Of these 5, two are of diagnostic importance in Creutzfeldt-Jakob disease, one is reduced in schizophrenia, and two are increased in multiple sclerosis. Synthetic peptides have been made to these peptides, and antibodies are being produced, in order to develop sensitive, rapid and accurate immunoassays for these newly characterized proteins.

Two-dimensional electrophoretic survey studies have been continued with various disease model systems. Brains from inbred mice strains have been studied, with a normal protein reference being established for common strains, in order to study neurological mutants that have been bred on a similar genetic background. Hamster brain proteins have been studied in normal, heterozygotic autosomal dominant circadian rhythm mutants and homozygous mutants Multiple protein alterations have been identified, and further investigations are in progress to define which of these proteins is associated with variations in circadian rhythm.

Further developments to advance the resolution and detection of proteins has resulted in the synthesis of a new crosslinker which allows the polymerization of gels with better physical strength, less hydrophilicity, and better protein separation by two dimensional gel electrophoresis. Its probable lower affinity for silver ions and its greater resistance to hydrolysis also delays the appearance of a background stain with ammoniacal silver nitrate.

To further the section's studies of mutational events in mitochondrial DNA we have developed a rapid method of DNA sequencing by employing the polymerase chain reaction and an amplification primer with a 5' ligand to provide for a simplified method preparing single stranded DNA which is easily sequenced by the Sanger technique. This procedure should greatly facilitate the study and diagnosis of human genetic diseases, both nuclear and mitochondrial.

Progress in adapting RNA:DNA duplexes for the rapid screening of genomic variations: mismatches, substitutions and deletions, has provided some preliminary evidence for heterogeneity in the human brain mitochondrial genomes. Earlier experiments had suggested that mutations in the mitochondrial genome were very rare events. However, if the section's preliminary results are confirmed mutational events in the mitochondrial genome may provide some explanations for the pathophysiology associated with certain diseases and aging.

Studies of DHEA level in patients with AIDs and Alzheimer's disease were stimulated by observations that high levels of DHEA may provide protection against viral illness. Investigations of the lowered DHEA levels found in AIDs and Alzheimer's diseases may provide information on whether these lowered levels are disease specific. A double cross-over clinical trial has been initiated to determine whether the administration of DHEA has a beneficial effect in Alzheimer's disease patients.

The section is continuing to develop automated methods for the analysis of the complex protein patterns that are obtained with the high resolution two-dimensional electrophoresis of human body fluids and tissues. Computer programs are being developed to provide for the rapid comparison of protein patterns in groups of gels from clinical studies to provide for the identification and scoring of polymorphic proteins and other protein alterations which may be of importance in disease states.

The ability to detect trace proteins found in disease states and then to determine their primary sequence has proven to be invaluable in the development of rapid diagnostic tests.

Patents Awarded and Pending:

Patent Pending: NIH # E-191-87 diagnostic test for Creutzfeld-Jakob Disease.

Patent Pending: Development of polyacrylamide gels which improve the separation of proteins and their detection by silver staining.

Patent Pending: Catalysts for polyacrylamide gel polymerization which improve the detection of proteins by silver staining.

Honors

Dr. C.R. Merril: Awarded the PHS Commissioned Corps Outstanding Service Medal "For Leadership in the development of new biochemical methods for the investigation of human diseases", 1988.

ANNUAL REPORT OF THE CLINICAL NEUROSCIENCE BRANCH

National Institute of Mental Health

October 1, 1987 - September 30, 1988

David Pickar, M.D., Acting Chief

INTRODUCTION

The Clinical Neuroscience Branch conducts an interdisciplinary research program in the neurosciences with a major emphasis on elucidating the basic brain mechanism(s) involved in the etiology, pathophysiology, and treatment of the major neuropsychiatric disorders. The Branch consists of four sections, 1) the Section on Preclinical Studies (Steven M. Paul, M.D., Chief), 2) the Section on Brain Biochemistry (Candace B. Pert, Ph.D., Chief), 3) the Section on Molecular Pharmacology (Steven M. Paul, M.D., Acting Chief), and 4) the Section on Clinical Studies (David Pickar, M.D., Chief). In addition, there are two units in the Section on Molecular Pharmacology and Preclinical Studies: 1) the Unit on Behavioral Pharmacology (Jacqueline N. Crawley, Ph.D., Chief); 2) the Unit on Molecular Neurogenetics (Dr. Edward I. Ginns, M.D.; Chief). The current research focus of the Branch is quite diverse and although an attempt is made to emphasize studies related to the basic etiologic (or pathophysiological) processes underlying neuropsychiatric disease, individual investigators are encouraged to work on any problem related to the basic mechanisms of brain function. In general, the major areas of investigation involve characterizing critical aspects of synaptic transmission including the various neurotransmitter and neuromodulator substances themselves as well as their biosynthetic and metabolic enzymes.

SECTIONS ON PRECLINICAL STUDIES AND MOLECULAR PHARMACOLOGY

Steven M. Paul, M.D., Chief

Members of both the Section on Preclinical Studies and Molecular Pharmacology are engaged in studying the basic mechanisms involved in chemical neurotransmission and particularly as they relate to the mechanism(s) of psychotropic drug action. The principle drugs under investigation include the minor tranquilizers and related sedative/hypnotic agents such as the benzodiazepines, barbiturates, short-chain alcohols, and volatile anesthetics; as well as various psychomotor stimulants, antidepressants, and antipsychotic agents.

One of the primary research interests of the Section has been the characterization of both the ligand binding and gating properties of the GABA receptor complex. Over the past several years, an in vitro method has been developed to study GABA receptor-mediated chloride ion flux in a subcellular

brain preparation (synaptoneurosome). Using this method, several members of the Section have characterized the time course and pharmacologic specificity of the desensitization process induced by both GABA agonists and barbiturates. In addition, a variety of endogenous and exogenous substances have now been definitively shown to bind to and/or effect the function of this receptor. For example, we have previously shown that two naturally-occurring steroid hormone metabolites 3 5 -dihydroprogesterone and 3 -tetradeoxycorticosterone are potent ligands, binding to the GABA receptor complex and activating it in much the same way as the anesthetic barbiturates. These steroids have recently been shown by Dr. Leslie Morrow to potentiate GABA and muscimol-stimulated chloride flux at nanomolar concentrations. Like barbiturates, they directly activate (i.e. in the absence of GABA) chloride ion flux at higher concentrations (100-500 uM). However, their ability to potentiate GABA at extremely low concentrations suggests that they may, indeed, be physiological regulators of this receptor. Studies are underway to determine whether, in fact, these steroids are released during physiological conditions such as stress or during the estrus cycle and/or whether they modify GABAergic function via this proposed mechanism. Previous work by Drs. Crawley, Glowa, and Mendelson have shown that at pharmacologic doses these steroids produce anxiolytic and hypnotic actions in animals. This has prompted a collaborative effort with the pharmaceutical industry to develop a novel hypnotic compound based on the structure-activity relationships which have been delineated.

Previous work by members of our group has demonstrated that ethanol potentiates muscimol-stimulated chloride flux at relatively low (pharmacologically-relevant) concentrations. Other laboratories have confirmed these observations in both synaptosomal and cultured neuron preparations. Moreover, it appears that the sensitivity of various animals (strains of mice, and/or rats) to ethanol is highly correlated with the ability of ethanol to potentiate GABA receptor-mediated chloride flux in membrane vesicles prepared from these animals. Recently, in collaboration with Drs. Eric Moody and Phil Skolnick, we have shown that volatile anesthetic agents can also interact with the GABA receptor complex. These anesthetics displace ^{35}[S] tertiary butylbicylphosphorothionate (TBPS) binding to the receptor and (like barbiturates) are capable of activating chloride ion flux in vitro. These data suggest that volatile anesthetics such as halothane and enflurane may have significant actions on the same receptor complex that anesthetic barbiturates have been shown to produce their anesthetic actions. In a related series of studies we have tested a variety of benzodiazepine receptor inverse agonists for their ability to alter the behavioral actions of ethanol. One such compound, an imidazobenzodiazepine compound (Ro15-4513) has been shown to antagonize some of the low to moderate dose effects of ethanol in mice and rats. Ro15-4513 (0.1 - 1 uM) blocks ethanol potentiated muscimol-stimulated chloride flux in synaptoneurosomes and at low doses selectively blocks the anticonflict and sedative/intoxicating actions of ethanol in rodents. Further experiments over the past year have demonstrated that the ability of Ro15-4513 to antagonize these behavioral effects of alcohol are mediated through the benzodiazepine receptor in that they are blocked by Ro15-1788 and CGS 8216. A series of other inverse agonists have now been tested with respect to their "anti-alcohol" properties. Neither, the partial or full inverse agonists tested so far, including FG 7142 or BCCE, were able to antagonize ethanol in these paradigms. These data suggest that Ro15-4513 has a somewhat different or unique interaction with the receptor, which mediates its "anti-ethanol" properties. These data have been extended by other

laboratories and Ro15-4513 has also been reported to antagonize the discriminative stimulus properties of ethanol (but not pentobarbital) as well as the self-administration of ethanol in two rodent paradigms. In related experiments, Dr. Emmanuelle Lestringant has demonstrated that ethanol administration to animals results in an increase in the binding of [^{3}H] ouabain to neuronal Na^+K^+ ATPase in several brain regions including the hypothalamus and brainstem. This effect is observed at pharmacologically-relevant doses of ethanol and suggests that ethanol can alter the activity of the neuronal sodium pump. Dr. Lestringant is now examining the functional consequences of the alteration in [^{3}2H] ouabain binding using ^{86}rubidium flux measurements in synaptoneurosomes.

A major question pursued over the past year concerns the effects of short-and long-term administration of sedative/hypnotic drugs (known to interact with the benzodiazepine/GABA receptor) on the functional activity of the receptor. Is the development of behavioral tolerance accompanied by a demonstrable change in either the number or function of the receptor? Using an in vivo binding technique to measure benzodiazepine receptors (developed in our laboratory by Dr. Stephen Deutsch, Ronit Weizman, and Avraham Weizman) we have demonstrated alterations in receptor number and affinity in vivo that are not apparent using in vitro techniques. For example, both acute and chronic swim stress dramatically alter the binding of [^{3}H]Ro15-1788 when measured in vivo but not in vitro. Previous studies in our laboratory have shown that acute swim stress increases the binding of [^{3}H]Ro15-1788 to brain benzodiazepine receptors, whereas chronic swim stress produces the opposite effect (i.e. reduction in binding in several brain regions). Moreover, Drs. Ronit and Avraham Weizman have demonstrated that the effects of chronic swim stress on [^{3}H]Ro15-1788 binding in vivo (e.g. 40% reduction in Bmax in hippocampus) are not observed in adrenalectomized rats; suggesting that glucocorticoid secretion (or some other adrenal product) may regulate receptor density in various brain regions. Recently, in collaboration with Drs. Ginns, Martin and colleagues our laboratory has isolated human cDNA clones for the α subunit of the benzodiazepine/GABA receptor complex. This subunit, which binds benzodiazepines, is widely distributed in various brain regions. Drs. Pascale Montpied, Anne Lingford-Hughes, and Sandra Cottingham are studying the levels of mRNA under a variety of experimental and pharmacologic conditions; using both in situ hybridization and Northern analysis. Preliminary experiments suggest that the expression of the subunit receptor gene can be altered by both drugs and environmental manipulation. Other studies are now underway in collaboration with Drs. Ginns and Martin to explore the structure and function of gene(s) coding both the α $\alpha\nu\phi$ β subunits of the $GABA_A$ receptor complex.

UNIT ON MOLECULAR NEUROGENETICS

Edward I. Ginns, M.D., Ph.D., Chief

The research within the Molecular Neurogenetics Units focuses on the role of proteins and their genes in the pathogenesis of neurologic and psychiatric disorders. Drs. Ginns, Martin and their research associates are studying candidate genes that may be involved in the pathogenesis of bipolar illness, schizophrenia and other neuropsychiatric disorders. These include neurotransmitter synthesizing enzymes (such as tyrosine hydroxylase and tryptophan hydroxylase), receptors (muscarinic and GABA), cofactors, developmentally regulated neuron and glial specific proteins and lysosomal

hydrolases. Families having multiple individuals affected with psychiatric disorders (schizophrenia and bipolar illness) are also studied for linkage analysis and identification of candidate genes. A major goal of this work is to relate the description of normal and abnormal protein synthesis, maturation, intracellular compartmentalization and degradation to the heterogeneity observed in clinical symptoms in these neurologic and psychiatric disorders.

Dr. Ginns and colleagues have demonstrated that there are multiple isozymes of human tyrosine hydroxylase, in contrast to the single species present in the rat. The human gene, including regulatory elements (including the cAMP motif) as well as the alternative splice sites responsible for generating the different human isozymes have been identified. Dr. Ginns and Dr. Martin, in collaboration with Drs. Paul and Rehavi have also used the baculovirus high-level expression system to produce large quantities of the isozymes for biochemical, kinetic, and structural analyses. Additionally, in collaboration with Drs. Paul and Cottingham, isozymes of human tyrosine hydroxylase have been transferred to fibroblast and other heterologous cell lines using retroviral mediated gene transfer, and the conversion of tyrosine to L-Dopa has been demonstrated. The usefulness of these and other cell lines as sources of L-Dopa following implantation or transplantation is being investigated.

Dr. Martin has continued to extend his structural studies of several bioactive groups of peptides. In particular, in collaboration with Dr. Zasloff (Childrens Hospital of Philadelphia) he has determined the amino acid sequence of several of the magainins (a new class of potent antimicrobial peptides initially isolated from frog skin). Preliminary studies suggest the presence of similar peptides in mammals. Isolation and characterization of the mammalian magainin-like peptides are in progress. Studies have also been initiated with Dr. Crawley to ascertain the active peptide region of galanin, information that will be used to design and synthesize suitable antagonists. In collaboration with Dr. Possani (University of Mexico) studies on the bioactive components of scorpion venoms have led to the identification, purification and determination of the amino acid sequence of several sodium and potassium channel toxins. The studies on potassium channel toxins complement those reports of the cloning of the cDNAs for these important ion channel proteins. As a resource person, Dr. Martin has also collaborated with many other investigators on the NIH reservation on the chemical sequencing of proteins and cloning their cDNAs, including angiotensin converting enzyme, liver aldose reductase, and liver glycoprotein esterase. Drs. Ginns and Martin in collaboration with Drs. Pert and Ruff identified the sequence homology between vasoactive intestinal peptide (VIP) and Peptide T. Dr. Martin continues to provide a large number of homogeneous Peptide T analogues for bioassay testing, as well as develop the design and perform analysis of clinical clearance studies for peptide T.

Using Gaucher disease (the most common sphingolipidosis and most frequent Jewish genetic disorder)as a prototype of inherited disease have both neurologic and non-neurologic phenotypes, the Unit's research continues to delineate the genetic and biochemical basis of the clinical heterogeneity seen within the disorder. Work within the Unit was the first to demonstrate single base mutations in the glucocerebrosidase gene. Researchers within the unit continue to identify mutations in the human glucocerebrosidase gene and

develop procedures useful for carrier testing and genetics counseling in both type 1 (non-neurologic) and in the neurologic forms (type 2 and type 3) of Gaucher disease. The study of structure (active site and intrachain disulfide bridges) of glucocerebrosidase, the enzyme deficient in Gaucher disease, remains a high priority in order to obtain a more complete understanding of the biochemical basis of clinical heterogeneity. Using the high-level baculovirus expression system, large quantities of recombinant active human glucocerebrosidase have been produced. This enzyme will be useful for both structural and therapeutic studies. In collaboration with scientists at the Whitehead Institute for Biomedical Research (Boston), tissue culture, animal transplantation, and transgenic animal research continue in order to develop animals models of inherited disorders and to determine the feasibility of retroviral mediated gene transfer as a therapeutic alternative in Gaucher disease.

SECTION ON CLINICAL STUDIES, NSB

David Pickar, M.D., Chief

The Section on Clinical Studies, Clinical Neuroscience Branch, conducts clinical and basic research pertaining to the pathophysiology and treatment of schizophrenia and other serious mental disorders. The Section conducts its research on the 4-East Nursing Unit of the NIH Clinical Center and in the ACRF.

We have developed a clinical strategy by which neuroleptic-induced alteration in dopaminergic function is assessed using longitudinal plasma measurement of the dopamine metabolite, homovanillic acid (HVA). We have observed in repeated experiments that neuroleptic drugs produce time-dependent decreases in levels of plasma HVA with significant reduction occurring after several weeks of treatment. The observed correlation between neuroleptic-induced reduction in plasma HVA levels and the antipsychotic effects of neuroleptic drugs suggests that slowly developing reductions in presynaptic activity of dopamine neurons may more closely be associated with the mechanism of action of neuroleptic drugs than receptor blockade. This step-wise view of neuroleptic action holds promise as a model for understanding the antipsychotic process and for developing strategies to augment neuroleptic response.

In further investigations of plasma metabolite models, we have administered the peripherally acting MAO-inhibitor, debrisoquin, in order to increase the relative CNS contribution to circulating levels of HVA. Debrisoquin administration enhances neuroleptic-induced increase in plasma HVA during the initial days of treatment; similar to findings in non-debrisoquin treated subjects, prolonged (weeks) neuroleptic treatment decreases levels of plasma HVA below baseline. Thus, support is gained for the use of plasma levels of HVA to provide a reflection of CNS dopamine activity in response to pharmacologic treatment.

We are currently studying the clinical and biochemical effects of the atypical neuroleptic, clozapine, a drug which holds promise for new treatment efficacy in schizophrenic patients who otherwise are poor neuroleptic responders. We

are applying the longitudinal plasma HVA strategy and PET methodologies to investigate underlying mechanisms of clozapine action.

The highly promising and productive outpatient follow-up study of schizophrenic patients who had previously participated as inpatients in our research program is ongoing under the direction of senior staff fellow, Robert Litman and our social worker, Judy Schreiber. This project holds great promise for delineating factors predicting outcome in schizophrenic patients. To date we have observed that specific symptom clusters predict specific levels of functioning. Analysis of biological data as predictors is in progress.

Dr. Litman has applied a sophisticated eye tracking technique to studies of schizophrenic inpatients/outpatients. Preliminary data suggests that eye tracking dysfunction correlates with "defect" symptoms and may provide a marker for frontal cortical dysfunction in schizophrenia. In another new initiative Dr. Mark Rapaport has been instrumental in applying neuro-immunologic techniques to patients with schizophrenia. Preliminary data suggests immunologic abnormalities in a subset of patients with schizophrenia. Ongoing studies focus on the clinical significance of these findings.

Ms. Judy Schreiber, in collaboration with Dr. Carlos Pato of the Extramural Research Program, NIMH, has pursued the development of pedigrees which have high prevalences of schizophrenia. One particularly promising family pedigree is now ready for RFLP analysis in collaboration with the Unit on Molecular Neurogenetics.

Dr. Eric Konicki has made important contributions to maintaining the high level of clinical research on the 4-East clinical research ward and has also initiated studies of characterologic disorder patients. Dr. Richard Owen has joined the Section on Clinical Studies and has already proven to be an asset by developing a new initiative in early onset schizophrenia.

Ms. Jean Colison has continued her fine work with our computerized clinical data base which is now extended to outpatient as well as inpatient research. Biological and clinical data are integrated and accessible. Ms. Andrea Hobbs has provided outstanding secretarial and administrative support for the Section and for the Branch in the current time of transition. The efforts of Ms. Colison and Hobbs as well as our fine research assistants have enabled the research environment for clinical investigation to be rich and productive.

UNIT ON BEHAVIORAL NEUROPHARMACOLOGY

Jacqueline N. Crawley, Ph.D., Chief

Dr. Crawley and members of the Unit on Behavioral Neuropharmacology continue to investigate the functional significance of neuropeptides coexisting within the same neuron as "classical" neurotransmitters, in the awake, behaving rat. The two cases of coexistence which were the focus of experiments this year were: 1) cholecystokinin (CCK) and dopamine (DA) in the mesolimbic pathway, relevant to new treatments for schizophrenia; and 2) galanin (GAL) and acetylcholine (ACH) in the septohippocampal pathway, relevant to new treatments for Alzheimer's disease. In addition to the existing rat

behavioral paradigms, two new approaches have been adapted by our laboratory for studying the role of endogenous neuropeptides in behavioral events: 1) in vivo microdialysis; and 2) in situ hybridization.

We have previously demonstrated a facilitatory modulatory role for CCK, which was found to potentiate DA-induced hyperlocomotion when microinjected into the medial posterior nucleus accumbens, the terminal region, and which was found to potentiate DA-induced hypolocomotion when microinjected into the ventral tegmental area, the cell body region. To investigate the role of endogenous CCK in mediating mesolimbic function, a behavioral paradigm was developed to induce hyperlocomotion without drug intervention. Turning off the room lights, and adding novel objects to the Digiscan open field, produced an increase in locomotor activity in untreated rats, and in rats microinjected with saline into the medial posterior nucleus accumbens. Microinjection of a CCK antagonist, proglumide, into the medial posterior nucleus accumbens, significantly reduced dark-induced hyperlocomotion. This data provides the first evidence that endogenous CCK may contribute to mesolimbic function.

Our first goal in establishing the in vivo microdialysis technique for continuously sampling of extracellular fluids is to measure the release of DA and of CCK from the medial posterior nucleus accumbens, while rats are engaged in dark-induced hyperlocomotion, as compared to normal locomotion, and as compared to the quiescent state. The steps toward this goal which have been successfully completed by Dr. Susan De Mesquita, visiting scientist on sabbatical in our laboratory, from Marshall University School of Medicine, include:

1. Design and implantation of microdialysis probe carrier, probe insertion, and shielding of perfusion tubing, to allow continuous dialysis and sample collection while the rat is actively exploring the Digiscan open field.

2. High pressure liquid chromatography assay for monoamines and their metabolites at picograms per 20 microliter samples (Dr. Ivan Mefford, NIMH, and members of the laboratory of Dr. Agu Pert, NIMH, provided training and guidance).

3. Collaboration with Dr. Marge Beinfeld, St. Louis University School of Medicine, for radioimmunoassay of CCK to measure picogram quantities in 30 microliter samples.

4. Demonstration of increased DOPAC and increased CCK in the medial posterior nucleus accumbens after potassium-induced depolarization.

5. Demonstration of increased DOPAC and increased CCK in the medial posterior nucleus accumbens after acute haloperidol treatment, i.e. postsynaptic blockade to increase mesolimbic neuronal firing rate and increase presynaptic release.

6. Recovery through the microdialysis probe (Carnegie Medicine Company, Sweden), of an average of 20% for DA, DOPAC, HVA, and 5-HIAA, acceptable for these studies.

7. Recovery through the microdialysis probe (Carnegie Medicine Company, Sweden) of an average of 1% for CCK, unacceptable for these studies.

Dr. De Mesquita is now repeating all experiments with push-pull cannulae, to assure that the problem with the microdialysis probes is really the poor recovery for peptides. We are also testing other plastics as semipermeable diffusion membranes. A longer range approach will be to establish the capillary zone electrophoresis technique in our laboratory, which allows assay of atomolar quantities of amino acids and femtomolar quantities of neuropeptides.

Our second series of experiments using microdialysis is focused on the question of whether, and which, neuropeptides have major effects on ventral tegmental neurons to increase or decrease release of dopamine from the terminal regions of the mesocorticolimbic pathway. Dr. Kirsti Laitenin, visiting student from the University of Kuopio, Finland, is microinjecting CCK, neurotensin, substance P, substance K, or oxytocin into the ventral tegmental area, and using microdialysis probes in the medial posterior nucleus accumbens to measure changes in DOPAC in this terminal region. These peptides were chosen because of their previously established differential behavioral actions when microinjected into the ventral tegmental area. Rats administered neurotensin show increased locomotion and increased DOPAC. Rats administered CCK show no changes in locomotion, but a small increase in DOPAC. Rats administered substance P show an increase in locomotion, and a large increase in DOPAC. Rats administered substance K show a large increase in locomotion, and a large increase in DOPAC. Rats administered oxytocin show increased grooming, and a decrease in DOPAC. This procedure will be pursued at other terminal regions of the mesocorticolimbic pathway, e.g. medial frontal cortex, to test for a correlation between anatomical pathways mediating behavioral events and DOPAC release, and the peptides which have the greatest modulatory actions on the mesolimbic pathway.

Our first goal in establishing the in situ hybridization technique in our laboratory was to investigate whether mRNA transcription for CCK is regulated in concert with, or independently from, mRNA transcription for tyrosine hydroxylase, in ventral tegmental and substantia nigra neurons where CCK and DA coexist. Because of the small size of these nuclei, in situ hybridization and quantitation, rather than northern blot quantitation, is the method of choice. Dr. Sandra Cottingham, PRAT postdoctoral fellow with Dr. Steve Paul and in our laboratory, learned the in situ hybridization technique from Dr. Scott Young, NIMH, and is using the quantitation software programs developed by Dr. Wayne Rasband, NIMH. Our first experiments examined mRNA for TH and CCK in rat ventral tegmentum and substantia nigra, after acute and chronic treatment with haloperidol or clozapine. An increase in TH mRNA was seen 24 hours after both acute and chronic haloperidol. Data are being analyzed for CCK mRNA after haloperidol, and for TH and CCK mRNA after clozapine, as compared to saline-injected controls.

We previously demonstrated that rats with ibotenic acid lesions of the nucleus basalis-medial septal area showed deficits in a t-maze delayed alternation task, which were reversed by intraventricular administration of 7.5 or 10 ug acetylcholine (ACH). Dr. John Mastropaolo, postdoctoral staff fellow in our laboratory, investigated the role of galanin, which coexists with acetylcholine in the septohippocampal pathway of the rat, and in both the septohippocampal pathway and the nucleus basalis-cortical pathway in primates and humans, on this working memory paradigm. GAL, 100-500 ng, significantly reversed the ability of ACH to improve performance in the lesioned rats, when

GAL was coinjected with ACH into the lateral ventricle. GAL also reversed the ability of ACH to improve performance in the lesioned rats, when coinjected into the ventral hippocampus. GAL had no effect alone in either lesioned or sham-treated rats. These data suggest that GAL acts as an inhibitory modulator of ACH in the septohippocampal pathway. Our behavioral data are consistent with biochemical studies from other laboratories, showing that GAL inhibits the release of ACH from the ventral hippocampus, and GAL inhibits carbachol-stimulated phosphatidyl inositol hydrolysis in the ventral hippocampus. In addition, in collaboration with Dr. Bill Egan, FDA, using NMR analysis, we have determined that ACH and GAL do not form molecular complexes in vitro, indicating that the GAL-ACH interactions are biologically based.

Taken together, these several lines of evidence raise the possibility that GAL serves as an inhibitory feedback mechanism in cholinergic pathways, which may be deleterious in the case of Alzheimer's disease, in which cholinergic neurons degenerate. Removing the GAL inhibition might partially restore cholinergic function in Alzheimer's disease, or might allow cholinergic agonist drugs to function more effectively, to restore some of the memory deficits associated with this disorder. Our laboratory is therefore extremely interested in developing and testing GAL receptor antagonists. In collaboration with Dr. Brian Martin, NIMH, and with Dr. Tamas Bartfai, University of Stockholm, Sweden, we are making fragments of the 29 amino acid sequence of GAL, and testing them in our behavioral paradigm, to determine the minimum sequence for biological activity. Dr. Mark Austin, postdoctoral staff fellow in our laboratory, and Teresa Podruchy, technician in our laboratory, have determined that the active sequences to date are GAL 17-23, GAL 18-29, and GAL 24-29. Inactive sequences are GAL 1-16, GAL 12-29, and GAL 25-29. If these findings are replicated in our laboratory, and are consistent with biochemical studies in the laboratory of Dr. Bartfai, then amino acid substitutions of the smallest active sequences will be made to design and test potential GAL antagonists.

Our second goal in establishing the microdialysis technique is to measure the release of ACH and GAL from the ventral hippocampus while rats are engaged in t-maze delayed alternation performance, as compared to general exploration of the t-maze, and as compared to the quiescent state. Dr. De Mesquita, in collaboration with Dr. Dom Vicchio and Dr. Al Yergey, NICHD, is developing a liquid chromatography-mass spectrometry assay for ACH, which may be sensitive enough to measure ACH released from the ventral hippocampus and collected in the microdialysis probe, without adding the cholinesterase inhibitor, physostigmine, to the perfusion solution. In collaboration with Dr. Ake Rokaeus, Karolinska Institute, Stockholm, we are measuring GAL released from the ventral hippocampus and collected in the microdialysis probe by radioimmunoassay, using Dr. Rokaeus' antibody against porcine GAL. As with CCK, the peptide concentrations appear to be close to the threshold for this assay, and the recovery appears to be very low. We are now trying push-pull cannulae for GAL recovery and ventral hippocampus assays.

Our second goal in establishing in situ hybridization in our laboratory is to investigate whether mRNA transcription for GAL is regulated in concert with, or independently from, regulation of mRNA for choline acetyltransferase in nucleus basalis and medial septal neurons. Baseline and lesion studies are being conducted in collaboration with Dr. Tomas Hokfelt, Karolinska Institute, Stockholm. A second coexistence of galanin, with norepinephrine in the

approximately 80% of locus cerulein neurons, may be relevant to memory, to stress responses, and to antidepressant drug treatments. Mark Austin and Sandy Cottingham, are performing in situ hybridization studies of mRNA for tyrosine hydroxylase and for rat galanin in rats after treatment with reserpine, and after swim stress. Neither TH nor GAL mRNA levels in the locus ceruleus appear to be changed after one day or three days of 15-minute swim stress. A shorter time between swim stress and sacrifice may be required in future experiments. Reserpine treatment increased TH mRNA in the locus ceruleus. GAL mRNA after reserpine treatment is now being analyzed.

Sandy Cottingham has completed a characterization of the Maudsley Reactive rat, developed as a "hyperemotional" strain based on open field responses. Sandy and our collaborators, Dr. Leslie Morrow and Dr. Cathy McAllister, have found no differences between Maudsley Reactive and Maudsley Nonreactive littermates on benzodiazepine-GABA-linked chloride ion flux, or on several parameters of immune responses. Sandy found no differences in levels of mRNA for GAD in GABA neurons, or of mRNA for the alpha subunit of the GABA receptor, in several brain regions and peripheral organs of Maudsley Reactive versus Nonreactive rats. While the Reactives showed the previously documented increase in freezing and defecation in an open field, Sandy found no differences in the strains in development of the "learned helplessness" syndrome, or in circulating corticosterone levels after shuttlebox performance. However, the Reactives performed poorly on learning a simple two-way shuttlebox avoidance task. Learning was normal in the Reactives on two t-maze learning tasks, indicating no deficits in general learning in the Reactives. The deficit in shuttlebox performance was prevented by pretreatment with diazepam, indicating that the Maudsley Reactive rats may have an increased responsiveness to stressful environmental events.

Annual Report of the Laboratory of Developmental Psychology

National Institute of Mental Health

October 1, 1987 through September 30, 1988

Marian Radke-Yarrow, Chief

Research in the Laboratory of Developmental Psychology is concerned with dev-velopmental processes in well functioning children and in children with major behavioral disturbances, and with development in supportive and dysfunctional environments. Projects now underway include studies of the psychological and biological effects of sexual abuse of children, studies of young aggressive children, patterns of continuities and discontinuities in children's adaptive and maladaptive behaviors, biobehavioral relations in early adolescence, and the development of children of unipolar and bipolar depressed parents and well parents. Longitudinal or follow-up approaches are generally being used. The time span of measurement varies. Because each of the research areas poses difficult problems of measurement, major investment in methodological issues has been necessary. It should be noted that the kinds of research questions being addressed have benefited from collaborative research efforts, within and across scientific disciplines.

Work of the past year in each of the major projects is briefly described. Reports in previous years have, in many instances, dealt with these same projects at earlier stages in longitudinal assessments. In those reports, research designs and methods have been described in some detail. This year's summary will emphasize mainly findings and new directions in the research program.

A. Dr. Frank Putnam, in collaboration with Dr. Penelope Trickett, is carrying out a study of sexually-abused female children (aged 6-15) and matched controls. The effects of sexual abuse on psychological and physiological maturation are assessed. Specific attention is given to dissociative symptoms and the development of inappropriate sexual and aggressive behaviors. Dissociative behaviors are measured by checklists and standardized scales, and physiological maturation is determined by physical examination and hormone levels.

B. As part of his research on multiple personality and other dissociative disorders, Dr. Putnam is using the Dissociative Experiences Scale (described in MH-02368-01), to assess the contribution of dissociative phenomena to the symptomatology of several psychiatric and neurological illnesses. In a sub-group of patients with eating disorders a high level of dissociative symptoms were found, which is correlated with a history of unresponsiveness to treatment and low urinary free cortisol levels. A comparison of epileptic patients and psychiatric patients with pseudoseizures indicates that patients with verifiable epilepsy have significantly lower levels of dissociation and depersonalization than to patients with pseudoepilepsy. These studies are continuing and additional studies using the DES in other clinical populations are underway. The psychophysiology of dissociative states of consciousness is being explored in a number of laboratories, in collaboration with other investigators. Visual and auditory averaged evoked responses, galvanic skin responses (GSR), 24-hour continuous EEG and circulating immune functions are being measured. These findings indicate that there is a discrete state-dependent psychophysiology

associated with dissociative reactions.

C. Behavior problems involving misconduct, aggression, and oppositional behavior are among the most common reasons for referrals of children to health services. In previous work (MH-02155) Dr. Zahn-Waxler and her associates found significant continuities in these behaviors over the early years of childhood, from ages 2 to 5. These findings were based mainly on parents' reports. The investigators have continued the research, obtaining child assessments based on observations of the children's behaviors with peers and with mother. The data from these sources confirm the earlier findings. These investigators then expanded their evaluations to include affective elements in the children's behavior which might add to an understanding of children's maladaptive and adaptive interactions. For example, failure to feel remorse after wrongdoing and express emotions appropriately characterize the older aggressive children. Among the interesting findings on affect are the indicators of feelings of guilt in very young children. More children of mothers with affective problems showed atypical patterns of guilt, showing either avoidance or exaggerated expressions of responsibility when confronting emotional distress in another person, particularly distress in the mother. Also, children differ in their abilities to communicate about their feelings. These differences were found to be significantly related to their mothers' communication and openness about emotions. Depressed mothers relative to well mothers were less able to explain and explore emotions with their children.

Further research on aggression and anger in young children is being initiated in a collaborative longitudinal study of mono- and dizygotic twins (with Drs. Robert Emde, University of Colorado, Robert Plomin, Pennsylvania State University, Joseph Campos, University of Illinois and Carolyn Zahn-Waxler) in which questions of heritability and rearing experiences in relation to children's aggression are of interest.

D. Research on central nervous system biochemical-behavioral interactions, particularly in relation to serotonin metabolism and aggressive/impulsive suicidal behavior is ongoing by Dr. Brown (Z01 MH 00183-03 BP). This research was initiated in the Laboratory of Biological Psychiatry, prior to Dr. Brown's joining this laboratory. A strong trivariate relation was found between cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5HIAA) and histories of aggressive and suicidal behaviors. Family instability (particularly alcoholism in a parent) during one's childhood was found to be significantly associated with suicidal behavior in adolescence, but family instability during childhood was not significantly associated with a history of aggressive/impulsive behavior. Collaborative work on aggression in children has begun with Drs. Zahn-Waxler and Cole.

E. Research on early adolescence has been carried out by Dr. Nottelmann and Ms. Inoff-Germain in collaboration with investigators in NICHD and at Pennsylvania State University. Pubertal development and behavioral development have been studied longitudinally following children over a one-year period. (See MH-02164 for design and procedures.) Aggressive behaviors increased with age, especially in boys. Aggressive behaviors observed in laboratory assessments of interaction with their parents correlated with parents' reports and self-reports of their general trait-like behavior. The pattern for girls was much less clear. Longitudinal evaluations of hormone level change across 12 months indicate that

overall stability, is higher for boys than for girls. There is no stability in testosterone levels in girls.) There is not only a positive association between hormone level and stage of puberty, levels are also associated with subsequent rate of pubertal development. Although the general maturational course is a gradual rise in hormone levels, there are many cases of substantial decreases. Often these decreases follow large increases in the prior 6-month period. These data are being examined for potential disequilibrating effects in emotional and behavioral functioning. Analyses of interrelations of hormones and behavior are in progress in further longitudinal analyses.

F. The longitudinal study of unipolar and bipolar depressed parents and their children, and a comparison group of psychiatrically well parents and their children is a major program of research in the Laboratory. It is a large clinical study (120 families) that provides an extensive data set on which the research of a number of investigators is based.

The objectives of this study are to understand the processes whereby parental depression affects the child's experience, facilitates or interferes with critical developmental progress, and interacts with particular inherent vulnerabilities or strengths in the child. By using a developmental approach, it is possible to investigate the interaction of constitutional and experiential factors in the child's functioning at successive maturational stages. (See MH 02207 for details of design and procedures.) Parents and offspring are assessed at three time periods in the children's development: in early, middle, and late childhood. During this past year, the second assessment phase has been completed and the third phase of measurement has begun. Analyses of many of the data from Time 1 and some of the measurement at Time 2 have been completed, thus beginning to bring together a comprehensive developmental picture of the component factors (parent illness and functioning, family conditions, maturational and constitutional child characteristics) and their relation to psychological-psychiatric child outcomes.

Impairments in the rearing behaviors of depressed mothers appear across a broad spectrum of dimensions. As a group, compared with well mothers, depressed mothers (a) are less likely to provide the child with a secure mother-child relationship; (b) manifest more anxious, non-facilitative involvement of mother with child (Bridges-Cline); (c) are more likely to engage in negatively evaluative and negative affective interaction with the child (Radke-Yarrow); (d) have impaired methods of controlling or regulating the child's behavior, at both age levels, and, in their control strategies, are adapting less to the changing developmental capabilities of the child (Kochanska); are more often exploitative of the child's empathy, and more likely to convey their own stress to the child when confronted with stressful situations (Radke-Yarrow & Zahn-Waxler). In addition to these differences in interactions and relationships, families with depressed and well parents show robust group differences in the amount of chronic stress and chaos in their lives, the depressed families being greatly disadvantaged (Richters). These group findings are a first level of analysis. They are being followed by more detailed and process-oriented investigations that take into consideration differences of the parents' depressive illness and that deal with the pattern or accumulation of impairments within families.

Just as parental impairments appear across a broad range of measures, there are also multiple impairments in offspring of depressed parents. The children of

depressed parents, relative to the children of well parents, more frequently have an insecure relationship with the mother (ages 1 1/2 to 3). The offspring of both unipolar and bipolar parents are likely to differ, as a group, from same-age offspring of well parents in affective qualities (Radke-Yarrow & Nottelmann). They are more likely to manifest dysregulated negative affect (i.e. frequencies of one or more standard deviations above the group mean). Daughters (ages 1 1/2 to 3) of unipolar mothers exhibit significantly more negative affect than daughters of bipolar mothers, who, in turn, exhibit more negative affect than daughters of well mothers. Among boys, in contrast, there are no group differences in expression of negative affect related to mother's diagnosis. School-age children of severely depressed mothers are more resistant to mother's regulation attempts (Kochanska). Children's behaviors in the face of unfamiliarity (of persons and places) show group contrasts. Children (ages 2 to 3) of bipolar mothers show the highest levels of exploratory activity and confident, animated approach to and engagement with an unfamiliar adult. Children of unipolar mothers show more cautious and less active exploration and engagement (Bridges-Cline). Based on mothers' reports (5 year olds), children of depressed mothers have more sleep problems (Nottelmann & Stoleru). Based on mothers' reports, offspring of depressed parents have more externalizing (acting out) behavior problems and also more internalizing (shyness, worrying) behavior problems. Some children are both shy and aggressive. Psychiatric evaluations (McKnew & Cytryn), based on a semi-standard interview with the children (5 years and older), also differentiate the groups: Significantly more children of depressed mothers than of well mothers show problems of some clinical concern. These problems involve a broad range of deviations compared with the children of well mothers. More of the children of unipolar and bipolar mothers show problems at both periods of measurement, at 5 to 8 years and at 8 to 11 years.

In addition to the reported findings on mothers and children, other analyses are in progress: (a) comparing the rearing behaviors of mothers who are in an episode of depression with the behaviors of depressed mothers who are not in episode at the times of observation (Richters & Radke-Yarrow), (b) examining caregiving behaviors within the family (Bridges-Cline), (c) investigating fathers' behavior (Richters & Wilson), (d) obtaining data on children's behavior in school (Sherman), (e) investigating the different environments of the two siblings in each family in relation to sibling characteristics (Nottelmann & Radke-Yarow), and (f) assessing the psychiatric status of the children in later childhood (Brown & Free).

In the current (third) assessment of the families, family interactions and relationships are updated, each parent's and child's psychiatric status is reevaluated, and physical and physiological assessments are made of each child. Longitudinal analyses, spanning childhood, will begin as the Time 3 assessments progress.

Laboratory Personnel

Joining the Laboratory this year are two Staff Fellows, Pamela Cole and Kathleen Free. Both are clinical psychologists with extensive experience in work with children. Dr. Gerald Brown, psychiatrist, has transferred to our Laboratory from the Biological Psychiatry Branch.

The Laboratory has received research support awards for specific projects, from the W. T. Grant Foundations for the study of the effects of sexual abuse of children and from the John D. and Catherine T. MacArthur Foundation for the study of the social and emotional development of children of affectively ill and well parents. Also, Dr. Marian Radke-Yarrow has been appointed by the MacArthur Foundation to organize collaborative research with investigators from other research institutions on family influences on children's development. Two areas will be emphasized: (1) developmental psychopathology in the context of the family and (2) the shared and nonshared environments of children in the same family.

This year investigators in the Laboratory of Developmental Psychology have received recognition from their scientific communities: Dr. Marian Radke Yarrow has been appointed Associate Editor of the Cambridge University Press Journal of Development and Psychopathology. Dr. Yarrow is also the recipient of the G. Stanley Hall Award of the American Psychological Association. Dr. Carolyn Zahn-Waxler has been appointed Associate Editor of the Journal of Developmental Psychology of the APA. Scientists in the Laboratory are active in the scientific community, presenting at national and international meetings, and serving on numerous boards, committees, and review panels in the scientific organizations of their disciplines.

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Two cortical visual pathways

Cortical tissue essential for visual perception extends far beyond the primary visual area, striate cortex, to include not only the prestriate regions of the occipital lobe but also large portions of the temporal and parietal lobes. Converging evidence from our neurobehavioral and neurobiological studies indicates that these extrastriate regions contain numerous areas that can be distinguished both structurally and functionally. Moreover, the multiple visual areas appear to be organized hierarchically into two separate cortical visual pathways, each having the striate cortex as the source of its initial input.

The first pathway consists of an occipitotemporal projection system. This pathway, which courses ventrally to interconnect the striate, prestriate, and inferior temporal areas, is crucial for the visual recognition of objects. Links between the occipitotemporal pathway and limbic structures in the temporal lobe as well as ventral portions of the prefrontal lobe appear to make possible the cognitive association of visual objects with other stimuli and other events, such as emotions and motor acts. The other pathway consists of an occipitoparietal projection system. This pathway, which courses dorsally to interconnect the striate, prestriate, and inferior parietal areas, is critical instead for the visual localization of objects. Links between the occipitoparietal pathway and both dorsal limbic and dorsal prefrontal cortex enable the cognitive construction of spatial maps, as well as the visual guidance of motor acts that may have been triggered initially by activity in the occipitotemporal pathway. In contrast to the occipitotemporal pathway, which remains modality-specific throughout its course, the later stations in the occipitoparietal pathway appear to receive convergent input from other modalities and so constitute polysensory areas.

To trace the flow of information through the two cortical visual pathways in detail, we have undertaken a series of anatomical studies using both anterograde and retrograde tracing techniques (e.g., amino-acid autoradiography, horseradish peroxidase histochemistry, and axonal transport of fluorescent dyes) in combination with electrophysiological recording. Our goal in these studies is to identify the multiple visual areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections.

Our results indicate that the occipitotemporal pathway begins with the striate projection to the second and third visual areas, V2 and V3, which in turn project to area V4. These three prestriate areas are arranged in adjacent

belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a representation of the contralateral visual field. Area V2 corresponds to cytoarchitectonic area OB, while V3 and V4 together correspond to area OA, exclusive of its dorsal part. The major output of V4 is to a widespread region within the inferior temporal (IT) cortex. Within posterior IT cortex, or architectonic area TEO, label was found primarily after V4 injections involving the representation of the central visual field, whereas within anterior IT cortex, or architectonic area TE, label was found after injections of any part of V4. Thus, mainly central field representations in V4 project to TEO, while both central and peripheral field representations in V4 project to TE.

Physiological studies have shown that TE has no discernible visuotopic organization. Rather, neurons in TE have very large receptive fields that nearly always include the center of gaze and frequently cross the vertical meridian into the ipsilateral visual field. Thus, a single neuron in TE can "see" an object no matter where it occurs in the field, which is in keeping with the crucial role this area plays in object recognition. Surprisingly, almost nothing is known about the properties of neurons in TEO. As a first step in studying these properties, we have begun to map TEO electrophysiologically. Thus far, we have found that TEO contains a crude representation of the upper quadrant of the contralateral visual field. Like V4, TEO forms an elongated dorsal-to-ventral band, and the representations of visual eccentricities seem to parallel those in V4. The representations of the upper foveal and parafoveal visual fields are located on the inferior convexity of the hemisphere, adjacent to area TE, while the representation of the upper periphery lies on the ventral surface of the hemisphere, adjacent to unresponsive cortex. An especially high percentage of receptive fields recorded in TEO include the fovea, which is consistent with the input this area receives from the central visual field representation of V4. Because lesions of TEO, unlike those of TE, produce impairments in pattern perception rather than in object recognition, a physiological comparison of TEO with TE should help in understanding the neural mechanisms underlying these functions.

Unlike the occipitotemporal pathway, the occipitoparietal pathway begins with striate, V2, and V3 projections to visual area MT, which is located in the caudal portion of the superior temporal sulcus, mainly within the dorsolateral portion of cytoarchitectonic area OA. MT projects to three additional areas located in parietal cortex, or cytoarchitectonic area PG. Thus, although MT receives inputs from areas belonging to the occipitotemporal pathway, its outputs appear to be directed mainly into the parietal lobe.

One projection zone of MT, area VIP, lies ventrally in the anterior two-thirds of the intraparietal sulcus, while the other two, areas MST and FST, are located on the medial bank and floor, respectively, of the superior temporal sulcus. To examine the role of these areas in visual function, we have recorded the electrophysiological properties of neurons within MT's projection zones and compared their properties with those of neurons in MT itself. Our results indicate that, like neurons in MT, a majority of those in MST and a third of those in FST are highly sensitive to the direction of stimulus motion but are insensitive to both the form and color of a visual stimulus. Compared

to neurons in MT, however, neurons in MST and FST integrate motion information over progressively larger portions of the visual field and respond selectively to more complex types of visual motion. Thus, MT and the areas to which it projects may constitute a cortical system for motion analysis.

To help identify additional components of this motion-analysis system, we have determined the targets of both MST and FST, which include not only widespread regions of the posterior parietal cortex but also several areas on the medial bank and floor of the anterior portion of the superior temporal sulcus. Thus, the cortical pathway for motion analysis seems to split into two components, a parietal and a temporal. Although lesions along the parietal component of this system are known to cause impairment in spatial perception, eye movements, and visually guided hand movements, the effects of lesions along the temporal component of the system remain to be explored.

Impairments in smooth pursuit eye movements following MST lesions are similar to those observed in patients with lesions at the junction of the parietal, temporal, and occipital lobes (PTO), suggesting that PTO in human cortex and MST in monkey cortex play similar roles in this function. Because neural damage in patients often includes the white matter, it is important to identify those fiber bundles in monkeys that interconnect the areas involved in smooth pursuit. We have found that the relevant white matter pathways consist of three different types of cortical fibers: 1) arcuate fibers, which course beneath the cortical mantle to interconnect striate cortex with MT, MT with MST, and MST with posterior parietal cortex; 2) the tapetum/major forceps, which is comprised of commissural fibers that pass through the splenium of the corpus callosum to interconnect MT and MST of one hemisphere with MT and MST of the opposite hemisphere; and 3) the internal sagittal stratum, which is the major subcortical fiber bundle projecting from MST to the dorsolateral and lateral pontine nuclei. Based on the effects of lesions on smooth pursuit, these corticocortical and corticosubcortical pathways can be divided into sensory, motor, and attentional/spatial systems. Evidence from clinical studies suggests that homologous systems exist in the human cerebrum.

Although MT plays a pivotal role in the occipitoparietal pathway, it does not provide the sole route by which visual information from striate cortex reaches the parietal lobe. Other potential pathways include those through additional visual areas located in occipito-parietal cortex. Our recent findings indicate that these areas receive inputs representing predominantly the peripheral visual field, which presumably reflects the importance of such inputs for spatial vision. We have also found a predominance of peripheral over central field inputs in the projections of V4 to area TF on the parahippocampal gyrus, suggesting that this temporal lobe region, like the parietal cortex, may have chiefly visuospatial functions. By contrast, the predominance of central field inputs from V4 to TEO in the posterior portion of the inferior temporal cortex presumably reflects the importance of these inputs for pattern perception.

To determine how the object and spatial information carried by the occipitotemporal and occipitoparietal pathways are integrated to yield a

unified percept, we have begun to investigate possible anatomical sites of interaction. Accordingly, we have made multiple injections of two different anatomical tracers into the lower bank of the intraparietal sulcus (following removal of the upper bank) and into the inferior temporal cortex, and then identified and compared the distributions of cells in extrastriate visual cortex projecting to these two destinations. Although cells projecting to temporal and parietal cortex were found to be located mainly in different areas, two areas contained cells projecting to both: V4 and the posterior bank and floor of the STS outside MT. In both V4 and STS, labeled cells projecting to the two destinations were intermingled, though the projection to parietal cortex was heavier from the peripheral than from the central field representation of V4. The laminar distribution of labeled cells suggests that V4 provides feedforward information to both temporal and parietal cortex, whereas zones within the STS may be a site for convergence of information from these regions. We are currently investigating whether prefrontal cortex is another possible site for convergence.

Recently, we have undertaken a collaborative study with members of the Laboratory of Neurosciences of the NIA to investigate the possible presence and location of separate visual pathways in human cortex for processing object identity and spatial location. In this study, regional cerebral blood flow was measured with positron emission tomography (PET) as subjects performed both an object identity and spatial location task. Areas activated more during the object than the spatial task were located in occipitotemporal cortex, whereas areas activated in the spatial but not the object task were located in superior parietal cortex. These results demonstrate the existence in humans, as in monkeys, of two distinct visual processing pathways, although there appear to be cross-species differences in their anatomical locations.

The occipitotemporal pathway and stimulus encoding

In any physical system, accurate knowledge of how its elemental units function is critical for modelling the mechanisms through which system properties arise. Anatomical delineation of the occipitotemporal pathway has opened the way to a study of the functional properties of its single neurons, and, with the knowledge gained, of how networks of these neurons give rise to visual perception and recognition. With this goal, we have studied how information about visual patterns is encoded in the responses of single visual system neurons. The key has been to develop a new approach in which single neurons are viewed as communication channels, so that techniques from statistics, signal processing, and information theory can be applied.

Experiments were carried out by presenting to the awake, behaving monkey visual stimuli constructed from basic signal elements while we recorded from single neurons in both the first and last stations of the occipitotemporal pathway. We found that information about these patterns was encoded in several (3-5), simultaneously transmitted messages in both stations. On the basis of these results, we have proposed a new model of the function of visual system neurons, viz, the multiple-filter hypothesis. The hypothesis states that neurons respond to visual stimuli as though they consisted of several, simultaneously active, spatial-to-temporal transforming or filtering

mechanisms. The filtering mechanisms are reflected in the independent activity patterns or temporal modulations of the neuronal spike train, which can thus be viewed as containing several messages about the stimulus multiplexed into the spike train. When this hypothesis was tested with a quantitative model, the model accurately predicted the temporally modulated responses of striate cortical complex cells to complex mixtures of basic stimuli.

Analysis of the messages showed that information about different stimulus features such as form, luminance, and duration, is encoded and transmitted in the multiplexed messages, and that these different stimulus parameters each influence the responses differently. Thus, information about each of these features might be decodable from the response of a single neuron. The potential separability of information about different stimulus features from a multidimensional neural response challenges a commonly held assumption that information about different features can only be decoded across a large ensemble of neurons. Further, it raises the possibility that a neural code might exist, and, indeed, we have found a potential structure for this neural code. When we examined the three most basic temporal response patterns to our basic stimulus patterns on a 3-dimensional graph, we found the response for each basic stimulus pattern lay in its own plane regardless of the feature's luminance or duration of presentation, and the planes for many different stimulus forms were each uniquely oriented. This suggests that there is a neural code that can be interpreted in terms of visual features. Decoding could be done locally by determining the stimulus feature plane that a response belonged to, a task that could require as few as three neurons (three points can uniquely determine a plane).

We have now examined the pathway from retina to primary visual cortex to find out where the temporal code originates. A rigorous analysis of the information transmitted showed that the fibers leaving the retina (the ganglion cell fibers) carry just as much information as the extraretinal neurons, but the former carry a substantially smaller proportion in the temporal modulation as compared to the response strength alone. Single units in all the regions we have examined carry about the same amount of information (3 bits/sec), so the change in the proportion of information found in the temporal modulation represents a redistribution of the information within the signal. This temporal modulation gradually becomes more prominent as successive processing stages are traversed.

Because our results showed that lateral geniculate neurons use temporal modulation, we applied our multiple-filter model to LGN neurons. After the multiple-filter model was trained with responses to our basic stimulus set, it accurately predicted the responses of LGN neurons to arbitrary patterns. This suggests that, at least early in the visual pathway, the multiple-filter hypothesis provides a conceptual model of neuronal function that can be used to quantitatively describe the stimulus-dependent activity of single neurons.

The occipitotemporal pathway and the analysis of color, contour, and shape

Now that we know the location, visuotopic organization, and connections of many of the extrastriate areas in the occipitotemporal pathway, we can trace

the transformation of visual information through them at the single neuron level. We began our physiological analyses in area V4, which plays a crucial role in the relay of visual information into the temporal lobe. We found that, as in striate cortex, some neurons in V4 are sensitive to object contours and low spatial frequencies, whereas others are more sensitive to textures and high spatial frequencies. Thus, the data on V4 suggest that within each visual area of the occipitotemporal pathway, the contours of object surfaces and the textures of object surfaces may be processed by separate populations of neurons. Surface color also appears to be processed by neurons within each of these visual areas. The results argue against the prevalent view that each area of the occipitotemporal pathway processes a different aspect of an object separately, such as color in V4 and depth in V2, and suggest instead that the different features of an object are processed in parallel within each area. At the same time, the results are consistent with our anatomical evidence indicating that the occipitotemporal pathway is organized as a serial hierarchy.

A hierarchical model predicts that the product of visual processing will become progressively more complex at each successive stage in the pathway. So far, our results in V4 support this prediction. In addition to sensitivity to the contour, texture, and color of a stimulus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. This responsivity to stimulus differences in the receptive field is due to a unique receptive-field structure of the neurons in V4: a small excitatory receptive field surrounded by a large, silent, suppressive zone. The surround zone is silent in that stimulation of it alone is without effect, but is is also suppressive in that it has properties antagonistic to those of the excitatory field and, hence, can suppress the response to an excitatory-field stimulus if the surround is stimulated in the same way. Thus, V4 neurons may play a role in separating figure from ground, a fundamental requirement for object perception. Now that we have a better understanding of sensory coding in V4, we will concentrate our future efforts on investigating the mechanism by which coding is controlled by cognitive factors such as attention.

The occipitotemporal pathway and selective attention

Our retinas are constantly bombarded by a welter of shapes, colors, and textures. Since we are aware of only a small amount of this information at any one moment, most of it must be filtered out centrally. Yet, this filtering cannot easily be explained by the known properties of the visual system. At each successive stage along the pathway from the striate cortex into the temporal lobe there is an increase in receptive field size. Many different stimuli will typically fall within these large receptive fields, and thus, paradoxially, more rather than less information appears to be processed by single neurons at each successive stage. How then does the visual system limit processing of unwanted stimuli? The results of our single-neuron recording experiments in visual cortex of trained monkeys indicate that unwanted information is filtered from the receptive fields of neurons in extrastriate cortex as a result of selective attention.

We trained monkeys to maintain fixation on a target while performing a task that required them to attend selectively to stimuli presented at one visual field location. Irrelevant stimuli were simultaneously presented at a location outside the monkey's focus of attention. When stimuli at both the attended and ignored locations were simultaneously present within the receptive field of a cell in either area V4 or the inferior temporal cortex, we found that the cell responded to stimuli only at the attended location. For example, if the cell was selective for red stimuli, it would respond well if a red stimulus appeared at an attended location but poorly or not at all if a red stimulus appeared at an ignored location even though it was still in the receptive field. Thus, we have shown for the first time that the processing of unwanted visual stimuli in extrastriate cortex can be blocked as a result of selective attention. Our most recent evidence indicates that attention can also be directed to a specific sensory dimension of a complex stimulus, such as its texture or shape, and that the neuronal processing of irrelevant information may thereby be reduced even further. We propose that it is these extrastriate neural mechanisms for selective attention that enable us to identify and remember the properties of a particular stimulus out of the many that may be acting on the retina at any given moment.

Not only do extrastriate neurons fail to respond to unattended stimuli, but also the magnitude of their response to attended stimuli depends on how much attention, or effort, the animal devotes to the stimuli. Extrastriate neuronal responses to a given stimulus are larger and more tightly tuned when the monkey is discriminating that stimulus from one that is very similar to it than when the monkey is discriminating it from one that is very different. Likewise, behavioral data indicate that the monkeys' discriminative abilities are improved when they are engaged in a difficult task. Thus, when an animal is challenged by a difficult task, it appears to "rise to the occasion" by concentrating its attention, two neural correlates of which appear to be enhanced responses and sharpened selectivity of the neurons that are processing the stimuli used in the task.

To identify the mechanisms by which cognitive state modulates cortical activity, we have begun to examine both extrastriate neuronal responses and the animal's performance in an attention-demanding task following lesions (or reversible deactivation) of structures known to have connections with area V4 and the inferior temporal cortex. Anatomical experiments in our laboratory indicate the most likely structures to be specific portions of the lateral pulvinar, posterior parietal cortex, and prefrontal cortex. Preliminary results from studies of the pulvinar and posterior parietal cortex indicate that these two structures have very different roles in the modulation of sensory processing by attention. Unilateral deactivation of the pulvinar severely impaired performance of a visual discrimination task when a distracting stimulus was present in the visual field, suggesting that the monkey could not focus its attention and thereby ignore the distracting stimulus. The ability of the monkey to switch attention from one location to another was unimpaired following the deactivation. Exactly opposite results were found following unilateral lesions of the posterior parietal cortex. Such lesions had no effect on the ability of the monkey to perform a color or form discrimination in the presence of a distracting stimulus, indicating that

there was no impairment in the ability of the monkey to focus its attention. However, the lesions did increase the time it took the monkey to switch its attention from one location to another. These contrasting effects of pulvinar and posterior parietal dysfunction support the notion that the mechanisms underlying selective attention involve a number of different components, each of which may be functionally associated with a different neural circut.

A visual cortico-limbic circuit and recognition memory

Monkeys that are shown a series of objects once will demonstrate that they recognize them as familiar several minutes later by consistently choosing them over novel objects or by avoiding them in favor of the novel objects, depending on the paradigm (delayed matching or nonmatching-to-sample, respectively). Thus, somewhere in the visual system the single presentation of a series of complex stimuli leaves traces against which a subsequent presentation of those same stimuli can be matched. If they do match, i.e. if the original neural traces are reactivated, there is immediate recognition of familiarity. The area in which the neural traces are first established appears to be area TE, since removals here but not elsewhere in the visual system abolish the animal's ability to recognize objects that it has seen once just a few seconds before. Apparently, area TE contains the traces laid down by previous viewing, and these serve as stored representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay or be renewed or even refined, while new traces are added to the store.

Significantly, area TE projects directly to the amygdala and indirectly to the hippocampus via perirhinal and entorhinal cortex. In our previous work we had established that at least part of the neural circuit necessary for storing the traces includes the cortico-limbo-thalamic system, which is actually composed of two largely independent systems arranged in parallel. One of these systems consists of the amygdala, amygdalofugal pathways, and the magnocellular portion of the medial dorsal nucleus (MDmc), and the other consists of the hippocampus, fornix, and anterior nuclei of the thalamus (Ant N). The evidence that these two systems operate in parallel comes from our finding that damage to the amygdalar and hippocampal systems at any stage (i.e. medial temporal lobe, limbo-thalamic pathways, and medial thalamus) causes a severe loss in recognition memory, but only when the two systems are damaged in combination. Damage to just one of the two leads to only mild recognition deficits, suggesting that either system can compensate for loss of the other, at least so far as recognition memory is concerned. Recent results indicate that combined but not separate removal of the orbital frontal and anterior cingulate cortices, which are related anatomically to the amygdalar and hippocampal pathways, respectively, will also produce a severe recognition deficit. Thus, the ventromedial prefrontal region appears to constitute yet another stage in the limbic memory system.

Recent anatomical evidence has indicated that the bed nucleus of the stria terminalis occupies an anatomical position within the amygdalar system that is comparable in some respects to the position occupied by the mamillary bodies within the hippocampal system. That is, just as the hippocampal formation

projects to Ant N both directly and indirectly via the mamillary bodies, the amygdala projects to MDmc both directly and indirectly via the bed nucleus of the stria terminalis. These particular relays between the medial temporal lube and medial thalamus are not normally critical for recognition memory, since combined damage to the two relays yields only a mild impairment. However, they might have an important role in the enhancement of memory by emotion, a possibility that we plan to explore.

Our experimental evidence indicating that combined damage to these two limbo-thalamic systems is necessary to produce a disorder in monkeys resembling the syndrome of global amnesia in man is consistent with most of the neuropathological evidence available on amnesic patients, including patients with temporal lobe resections and those with Korsakoff's disease. One piece of clinical evidence does not seem to fit this view, however, and supports instead the view that damage to the hippocampal formation alone is sufficient to produce the syndrome. The evidence comes from amnesic patients with diseases of the posterior cerebral artery, which is known to provide the blood supply of the hippocampus but not the amygdala. To examine this issue experimentally, we occluded the posterior cerebral artery in the monkey and found a substantial visual recognition loss in several animals, with scores averaging 20% below those of normal controls. These animals had bilateral infarctions confined almost entirely to the hippocampal formation and parahippocampal gyrus, and then only to restricted portions of these structures. Indeed, the only subfields of the hippocampus damaged in common in these cases were CA1 and CA2. Paradoxically, the memory loss found in these animals with only partial bilateral hippocampal damage was significantly greater that that found in animals with total bilateral ablation of the hippocampal formation, whose scores averaged only 10 percent below those of normal controls. Consideration of the possible mechanisms accounting for this paradoxical finding may be important in understanding the memory deficits found in amnesic patients with partial damage to the hippocampus resulting from hypoxic ischemic episodes as well as from cerebrovascular accidents.

The cholinergic system and recognition memory

In the neural model that we have proposed to account for recognition memory, a stimulus leaves a trace in the sensory modality's higher order processing stations whenever that stimulus activates the cortico-limbic pathway described above. This hypothetical circuit is incomplete, however, because it does not specify the link through which the limbic structures reactivate the sensory cortical areas that activated them. One possible candidate for this missing link is the basal forebrain cholinergic system. Three major lines of evidence support this view. First, cholinergic agonists and antagonists have long been known to influence many forms of memory in many species, and in a number of studies of our own we have found that the same holds true for various forms of memory in the monkey. Moreover, we have recently found that scopolamine, a muscarinic-receptor blocker, prevent the storage of information at a very early stage, i.e. between 0 and 1 second after stimulus offset, without affecting perception, i.e. 0 seconds after stimulus offset. This finding suggests that scopolamine blocks primary memory and, thus, that binding of acetylcholine to muscarinic receptors is necessary for any entry into a memory

store. In a related study, we have now mapped the distributions of nicotinic and muscarinic cholinergic receptors in the adult monkey brain. Both types of receptors are found in all cortical areas, but the muscarinic receptors are more widely distributed across the layers of a given field and have a wider variety of laminar labeling patterns across fields than nicotinic receptors. Nicotinic receptors are found predominantly in the deep part of layer III, where incoming thalamic and corticocortical afferents terminate densely, in keeping with the pattern characterizing the forward projections of the sensory processing pathways. Muscarinic receptors, by contrast, are commonly found most densely in the upper and lower layers of the cortex, a pattern that suggests a muscarinic role in the central modulation of sensory processing via the backward projections of those same pathways. The receptor distribution patterns thus appear to fit the notion that the mnemonic effects of the anticholinergic agent scopolamine are exerted through a blockade of the central modulating system.

The second line of evidence favoring the cholinergie hypothesis involves recent neuropathological studies in patients with Alzheimer's disease, who often show a marked memory loss as one of their earliest symptoms. Postmortem examination of such cases revealed selected cell loss in both the nucleus basalis of Meynert (nbM), the major source of cholinergic input to the cerebral cortex and amygdala, and the nuclei of the medial septum and diagonal band of Broca (ms/dbB), the major sources of cholinergic input to the hippocampus. This cell loss is accompanied by markedly decreased cortical and limbic levels of choline acetyltransferase and acetylcholinesterase, enzymes involved in the synthesis and metabolism of acetylcholine. In experiments performed in collaboration with investigators from The Johns Hopkins Medical School, we produced recognition memory impairments in monkeys by damaging the basal forebrain with a neurotoxin. The monkeys with the most severe memory impairment had nearly complete destruction of the basal forebrain cholinergic nuclei as well as a 60-90% loss of choline acetyltransferase activity across most of the cortex. However, there were only small decreases of this critical enzyme in area TE, the cortical sector that is so important for visual recognition memory. The results suggest the interesting possibility that the visual recognition impairment resulted from cholinergic denervation not of the cortex but of the limbic system. This possibility would also account for the finding that only combined damage of nbM and ms/dbB yielded impairment, since only such combined damage would result in cholinergic denervation of both the amygdala and hippocampus.

The third line of evidence in support of a cholinergic mechanism comes from a study that was aimed at providing a more complete picture of the anatomical relations between the limbic system and the basal forebrain. In this investigation we found that the amygdala and hippocampus project mainly to the same parts of the basal forebrain that innervate them, namely, the nbM and ms/dbB, respectively, with little overlap between them. Interestingly, because the visual system itself does not project directly to the basal forebrain, a relay through the limbic system would appear to be essential if the visual system is to activate the cholinergic mechanism.

An extremely puzzling aspect of the recognition ability of the animals with basal forebrain lesions is that it recovered fully after several months of testing. Because the cortical markers of cholinergic activity clearly did not recover during the same period, the results raise the possibility that other neurotransmitter systems may have compensated for the loss of the cholinergic system. Results from other research on cortical plasticity suggest a compensatory relationship between the activities of norepinephrine and acetylcholine. Although it is a completely open question as to whether norepinephrine is the neurotransmitter involved, loss of neurons in the locus coeruleus, the major source of noradrenergic input to the cortex, has been reported in cases of Alzheimer's disease. Another candidate is arginine vasopressin, an endogenous neuropeptide that has been reported to be decreased in Alzheimer's disease. We have recently shown that although vasopressin alone does not affect recognition memory, it does block scopolamine-induced impairment.

In conjunction with these experimental manipulations of the basal forebrain cholinergic system, the goal of which is a better understanding of the nature and neurobiological basis of the cognitive losses associated with Alzheimer's disease, we have begun to examine the cognitive losses associated with normal aging in the monkey. Like the neurotoxic-lesion study, this one too is being performed in collaboration with investigators from The Johns Hopkins University Medical School. Our initial results demonstrated a moderate but systematic decline in the recognition memory of monkeys from early adulthood (3-6 yrs), through middle age (14-17 yrs), to old age (26-30 yrs), as well as an age-related impairment in the type of spatial memory measured by the classical delayed response test. Since there was no correlation between the object recognition and spatial memory deficits in the aged animals, and since the two abilities are known to depend on largely different neural substrates, it is likely that multiple neural systems are vulnerable to the effects of aging and that the vulnerabilities differ from animal to animal. In the future, we plan to test this hypothesis directly by microscopic examination of the tissues in question.

Molecular mechanisms of neural plasticity

It is likely that release of acetylcholine into the synapses of neurons in the sensory processing pathways initiates a cascade of cellular neurochemical events that leads to the strengthening of the synapses. As a result, many of the neurons whose signals have just represented a sensory stimulus may become linked together in a cell assembly that serves as the stored representation of that stimulus. One cellular process that has become a strong candidate in the search for molecular mechanisms underlying neural plasticity is the phosphorylation of a protein band, F1. This protein band was found by investigators at Northwestern University to increase its rate of phosphate incorporation in the hippocampus both after memory formation and after long term potentiation. In collaboration with these investigators, we have found a protein in the cerebral cortex of the monkey that appears to be homologous to F1 on the basis of molecular weight, isoelectric point, two-dimensional phosphopeptide maps, and phosphorylation by exogenous, purified protein kinase C. To determine whether regional phosphorylation levels of this protein might

be related to information storage, we examined protein phosphorylation along the length of the occipitotemporal visual processing pathway. We found that phosphorylation levels of protein F1 increased in a gradient along the pathway, with levels in rostral inferior temporal and entorhinal cortices approximately ten times those in striate cortex. F1 phosphorylation could thus constitute one mechanism for the cortical storage of visual memories, and further collaborative studies with the investigators from Northwestern University are being planned to examine this possibility.

A cortico-limbic pathway for tactual object recognition

The work described above elucidating a cortico-limbo-neuromodulatory system for visual perception and memory has led to the search for analogous systems in other modalities. In fact, analogous anatomical pathways have been tentatively identified in all of the sensory modalities, but, because of our recent finding that combined amygdalo-hippocampal lesions produce severe recognition loss not only in vision but also in somesthesis, particular attention has been paid to the pathway in this modality. Also, anatomical studies on the somatosensory system have been supplemented with electrophysiological studies in order to permit functional comparisons with the memory circuit in vision.

Anatomical tracers were injected into the hand representations of physiologically identified somatosensory cortical fields in the postcentral strip and within the lateral sulcus, and the patterns of connections between fields were assessed. Applying principles previously worked out in the visual system regarding direction of information flow based on analysis of laminar projection patterns, and from our own work with combined lesion and recording studies, we have started determining the direction of flow of somatosensory information. Starting from the fields comprising primary somatosensory cortex in the postcentral strip, information is relayed along two pathways, one running dorsally through area 5 and then to area 7, and another coursing ventrally to SII and then onto the insula before reaching the limbic structures of the temporal lobe. This latter pathway is believed to be analogous to the ventrally directed object recognition pathway in the visual system.

In the course of identifying this somatosensory cortico-limbic pathway, we resolved a longstanding difficulty for our serial cortical processing hypothesis, which predicts that the postcentral strip and SII cortex process information in sequence. It was supposed earlier that these two cortical regions were both major projection zones of the ventroposterior nucleus, the primary somatosensory relay of the thalamus, implying that the two cortical areas received and processed information in parallel. Two major new findings support our view that SII receives its major source of activation from the cortical fields in postcentral cortex as opposed to the thalamus. The first is that following injections of tracers in SII cortex, very few labeled cells are found in the ventroposterior nucleus, demonstrating the lack of a major output from this portion of the thalamus to SII. The other finding is that following removal of the somatic fields comprising postcentral cortex, neurons in SII are no longer responsive to somatic stimulation, indicating the lack of

a driving input directly from the thalamus. The two findings together strongly support our suggestion of a sequential corticocortical processing pathway for somsthesis. New physiological and behavioral investigations are being directed at the insula, since our anatomical data point to this cortex as constituting the next station in a cortico-limbic pathway for tactile recognition. In addition, we have begun some neuroanatomical studies to clarify the connectional relations of the somatic areas comprising the dorsal pathway.

Another, unexpected result from the foregoing electrophysiological work was the finding that the SII region undergoes major functional reorganization following removal of portions of postcentral cortex. As indicated above, removal of all the representations of a body part in postcentral cortex (i.e. including its maps in 3a, 3b, 1, and 2) results in the failure to record somatically driven responses in the representation of the corresponding body part in SII. Interestingly, the SII tissue in question does not remain silent; instead, representations of different body parts in the adjacent portions of SII expand to occupy the partially deafferented cortical zone. For example, following a lesion of the postcentral representation of the hand, there is a greater probability of recording responses in SII to stimulation of the foot. Indeed, the areal extent of the foot representation increases to occupy most of the former hand region (a distance of 5 or more millimeters of cortex). These findings provide evidence for a previously unrecognized degree of cortical plasticity in adult primates. The results thus require major revisions of current theories, which tend to confer static properties on cortical maps.

Amygdala, hippocampus, and associative memory

According to our neural model, once the trace or representation of a stimulus has been stored in the higher order processing stations of any given modality, that stored trace can enter into association with the stored traces of other stimuli and other events, thereby providing the stimulus with associative meaning. As has been indicated, the amygdala and hippocampus, as well as their separate efferent pathways and separate thalamic targets, make approximately equal contributions to recognition memory, presumably reflecting their roughly equal contribution to the cortical storage of stimulus traces. In the case of associative memory, however, our results indicate that the amygdala and hippocampus make very different contributions.

In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two objects visually after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, whereas the performance of the amygdalectomized monkeys fell to chance.

Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this task, the animal was required to remember on the test trial where on a three-well tray each of two different objects had been presented on the acquisition trial. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance.

The results of these two complementary experiments indicate that, although both the amygdala and hippocampus are critical for certain forms of associative memory, their roles are totally different. Many further analyses along the lines of these experiments will of course be necessary before the selective associative memory functions of the amygdala and hippocampus can be identified with confidence. For example, the association of an object with an affective state, such as fear or pleasure, appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view has been obtained in an experiment showing that one-trial object-reward association is impaired more by amygdalar than by hippocampal lesions, although neither deficit approaches in severity the one produced by the combined removal of these two structures. By contrast, because of the important contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

In one new study that is currently underway, monkeys are being trained on a visual-visual associative memory task. The finding of impairment after amygdalar but not after hippocampal ablations would imply that the amygdala is critical for stimulus-stimulus associations in general and not just for those that cross modalities. The opposite outcome would suggest, in contrast, that the amygdala is critical for crossmodal associations only and that the hippocampus is responsible for all intramodal associations, with those between object quality and place constituting but one example. Preliminary results are inconsistent with both of these possibilities, and indicate instead that both the amygdala and hippocampus contribute to this kind of associative memory.

Nonlimbic structures and habit formation

On all of the memory tasks that have been described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid firgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, amnesic animals with the combined amygdalo-hippocampal removals learned to discriminate a long list of object pairs even though the list was presented

only once every 24 hours. The same result was obtained in amnesic animals with combined orbital frontal and cingulate lesions. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals.

Soon after discovering this phenomenon in tests with objects, we found that the same dissociation, i.e. impaired recognition but spared discrimination learning despite 24-hour intertrial intervals, holds for pictorial stimuli as well. This paradoxical success in the presence of severe cognitive memory loss implies the existence of a powerful learning and retention mechanism outside the limbic system.

We have since performed additional experiments to characterize further the essential difference in function between the limbic and nonlimbic mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from cognitive memory, we have labelled it 'habit formation'.

Another behavioral paradigm that may provide a measure of the ability to acquire habits is delayed nonmatching-to-sample (DNMS). Although combined amygdalo-hippocampal removals in macaques severely impair their performance on DNMS when delays between sample and choice exceed about 10 seconds, they can master the task with shorter delays. Such mastery cannot depend on the formation of specific visual discrimination habits, because (a) a different pair of objects is used on every trial and (b) within a trial, the reinforcement contingencies for responses to the sample object are inconsistent. To master the task in the absence of the limbic system, the animal must be able to learn a rule, which requires, in turn, (i) suppression of specific stimulus-response habits, (ii) abstraction of sameness and difference from specific stimulus quality with the aid of immediate memory, and (iii) formation of a stimulus/difference-response habit. We have now found that if inferior prefrontal lesions (which produce a moderate DNMS impairment by themselves) are added to amygdalo-hippocampal lesions, monkeys lose the ability to perform DNMS even when the delays are less than 10 seconds. This finding suggests that the inferior prefrontal cortex serves one or more of the processes described above needed for rule learning, and that it does so by mediating a complex set of interactions between the inferior temporal cortex and the neostriatum, with both of which the inferior prefrontal cortex is directly connected. These data open up a new chapter in frontal lobe research, namely, the role of the prefrontal cortex in the formation of complex habits.

Because the occipitotemporal pathway is known from behavioral evidence to form the initial part of the system underlying visual habit formation, and because other behavioral evidence suggests that the basal ganglia could also play a

role, we have recently begun to explore the projections from the occipitotemporal pathway to the neostriatum. So far, we have found that areas TE, TEO, and V4 all project to the tail of the caudate nucleus and to the ventral portion of the putamen. This arrangement contrasts with the pattern of projections from the occipitotemporal pathway to the limbic system, which arise from TE only. The presence of direct projections to the striatum but not to the limbic system from areas V4 and TEO may explain the ability of monkeys with TE lesions to acquire visual habits but not cognitive visual memories. Our aim now is to identify the structures to which the visual portions of the neostriatum project. Our present findings indicate that the efferent projections of both the tail of the caudate nucleus and ventral putamen are confined to the globus pallidus (GP) and substantia nigra, pars reticulata (SNr). Because the GP and SNr are known to project via the thalamus to the dorsolateral premotor and supplementary motor cortex, respectively, these cortical regions may represent further stations in the neural circuit of the postulated habit formation system. By delineating the wiring diagram of this system, we hope to identify structures that we can target for interventional neurobehavioral studies.

Ontogenetic development of cognitive memory and habit formation

Our evidence from studies in adult monkeys suggests that cognitive memory and habit formation are two qualitatively different retention processes based on separate neural mechanisms. Our studies of behavioral development in infant monkeys provide complementary evidence by suggesting that these two systems are developmentally dissociable, in that the limbic memory system appears to mature considerably later than the nonlimbic habit system. A similar delay in the maturation of the limbic memory system has been demonstrated recently in human infants. Our goal is to pursue studies in both monkeys and humans to determine how recognition memory measured by preferential looking differs from recognition memory measured by problem solving. This will help determine which capacities of the memory system appear late in ontogenetic development and, by implication, whether the phenomenon of infantile amnesia might be due to the absence of a fully functional cognitive memory system in early childhood.

To see how cognitive memories and habits develop in animals with early brain damage, we have prepared monkeys with neonatal removal either of the limbic system (i.e. combined amygdalo-hippocampal removals) or of area TE. With regard to habit formation, the results so far indicate that, whereas female infant monkeys can form a set of visual discrimination habits almost as quickly as adults, male infants are significantly retarded. In addition, whereas limbic lesions in both infants and adults leave habit formation intact, neonatal ablation of area TE impairs the learning of female but not of male infants, even though monkeys of both sexes are impaired when the lesions are made in adulthood. These data suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males, presumably because, at this age, area TE or some connected region is more fully developed in females than in males. This sexual dimorphism seems to be dependent on the high testosterone levels found in male infants before and shortly after birth, because a significant correlation

appeared between their testosterone levels and learning scores (the more testosterone the poorer the performance), and also because orchiectomy in male infants speeds their rate of habit formation whereas dihydrotestosterone in ovariectomized females retards their rate of habit formation.

With regard to formation of cognitive memories, the infants with limbic lesions are severely impaired at 10 months and 2 years of age, whereas those with TE lesions show significant and permanent functional sparing at both ages (compared to adults given TE lesions). The results thus point to a greater compensatory potential after neonatal cortical than after neonatal limbic removals and are consistent with the notion that association areas of the cortex are less mature at birth, and may thus possess greater plasticity, than limbic structures. Direct evidence of neocortical immaturity in the macaque has been provided by our recent behavioral studies showing that cortical areas (such as PG, TF, TEO, and STP) normally connected to limbic structures, but not involved in object recognition in adulthood, may assume a critical role in this memory function when area TE has been removed in early infancy; and by our neurobiological studies showing that (a) the distribution of both opiatergic and cholinergic receptors is adult-like at birth in subcortical structures and allocortical areas but is not yet fully developed in neocortical areas, particularly the association cortex, and (b) adult levels of metabolic activity in visual association cortex and particularly area TE are not reached until about 6 months of age. These behavioral and neurobiological findings suggest that the relatively poor recognition ability of normal neonates is due more to slow maturation of the cortical association areas than to neonatal immaturity of the limbic system.

We have also investigated whether the combined amygdalo-hippocampal removals in infant monkeys lead to socio-emotional abnormalities in the developing monkey. At 2 months of age, animals with combined limbic lesions had more temper tantrums when first placed in the novel cage, showed more passive behavior, and manipulated objects less than the controls. At 6 months, the same animals displayed a more striking pathology, namely, lack of social contact, extreme submissiveness including active withdrawal, and gross motor stereotypies. This syndrome was fractionated by partial limbic lesions, in that only increased locomotion and decreased manipulation were found after amygdalectomy, whereas only increased withdrawal from social contacts was found after hippocampectomy. By contrast, the animals with area TE lesions exhibited no significant abnormalities, except for decreased finger-sucking. The developmental time-course and the nature of the disturbances seen in animals with combined limbic lesions resemble those found in autistic children. This finding, together with the recent report of neuropathology in the amygdala, hippocampus, and cerebellum in each of three autistic subjects, provide evidence that early dysfunction of the limbic system may be one cause of infantile autism. In addition they indicate that the amygdala and the hippocampus are each components not only of a limbo-thalamic system serving cognitive functions but also of a limbo-hypothalamic system serving emotional functions. Though much more testing over a much larger time course is necessary, it is becoming clear that the same neonatal damage that leads to a severe cognitive memory disorder can also have extremely serious consequences for personality and social development, in part because the cognitive memory disorder is present from infancy onward, but also because of the direct effect of the limbic lesions on mechanisms of emotionality.

Summary

Through combined use of behavioral and neurobiological methods, we are beginning to discover some of the general principles along which the primate forebrain is organized to serve perception, attention, recognition, and recall, as well as some noncognitive learning processes.

Perception. Each primary projection area in the cortex seems to be the source of two multisynaptic corticocortical pathways. Both pathways are composed of several cortical areas that are arranged hierachically, one pathway being directed dorsally to the frontal motor system, the other ventrally to the temporal limbic system. Before reaching the motor system, the dorsal pathways from the several modalities converge in polysensory areas, which are critical for spatial perception and motor guidance. The ventral pathways, by contrast, remain modality specific throughout their course and are important instead for perception of objects or stimulus quality and, ultimately, for triggering the motor response.

Attention. In successive stations of each sensory pathway, single neurons carry messages from progressively wider sensory fields but about progressively more specific stimulus configurations. These messages are conveyed not simply by discharge frequency but by means of a temporal code, which appears to contain several simultaneous but independent messages about a stimulus, such as its form, intensity, and duration. Selective attention and attentional effort, reflecting central influences on sensory processing, can markedly alter the stimulus messages sent by individual sensory neurons, effectively reducing their receptive fields or narrowing their stimulus filters, or both.

Recognition. Stimulus recognition depends not only on attentionally filtered stimulus processing along the ventral cortical pathway but also on storage of a central representation of that stimulus (in Hebbian terms, formation of a cell assembly) largely, though not exclusively, in the ventral pathway's last station, located in the anterior temporo-insular region. This region projects to the amygdala and, via rhinal cortex, to the hippocampus, and these three limbic structures project in turn to the medial and midline thalamus, with further connections to ventromedial prefrontal cortex. All three of these cerebral regions (limbic, thalamic, and prefrontal) project to the basal forebrain cholinergic system and indirectly to other modulatory neurochemical systems, which innervate the entire cortical mantle. It is hypothesized that a cell assembly representing a configurational stimulus is formed only if this cortico-limbo-neuromodulatory-cortical circuit is activated. The resulting transmitter release may trigger protein phosphorylation and gene expression, to yield increased synaptic efficacy among some of the cortical neurons in the ensemble that just participated in conveying a stimulus message, and thereby form a stable cell assembly representing that stimulus. The slow ontogenetic development of cognitive memory ability, and, by implication, the phenomenon of global amnesia for the experiences of infancy, are probably the result of a slow development of the feedback mechanisms that are needed for the formation of cell assemblies.

Recall. Once a cell assembly has been formed, it can enter into association with other cell assemblies, thereby providing a stimulus with meaning. Cell assemblies appear to be linked not directly, however, but only indirectly via limbic structures (in Hebbian terms, a phase sequence). Thus, crossmodal stimulus-stimulus associations, as in recalling how an object looks by feeling it, appear to depend on cortico-amygdalo-cortical interactions, while stimulus-affect associations, as in fear, probably depend largely on cortico-amygdalo-hypothalamic interactions. By contrast, stimulus-place associations, as in recalling where objects are located, seem to depend on cortico-hippocampo-cortical interaction; in this case the hippocampus may act as the link between the ventral stimulus-recognition pathway and the dorsal spatial-perception pathway. Finally, stimulus-act associations could depend on interaction, via the limbic system, between the ventral and dorsal pathways within the lateral prefrontal cortex, which interacts in turn with the frontal motor system.

Habit formation. Destruction or disconnection of the limbic memory system does not affect all forms of learning and retention. At least one noncognitive form, which has been labeled habit formation, remains intact, presumably reflecting the operation of a powerful cortico-nonlimbic system for learning and retention. This system can mediate the acquisition not only of specific stimulus-response connections, probably through sensory-neostriatal interaction, but also of rules derived by abstracting stimulus relations from specific stimulus quality. Such rule learning appears to depend on a complex set of interactions between the relevant sensory pathway, the neostriatum, and the ventral prefrontal cortex.

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Annual Report of the
Laboratory of Psychology and Psychopathology
National Institute of Mental Health

October 1, 1987 to September 30, 1988

Allan F. Mirsky, Ph.D., Chief

This report summarizes the eighth year of activity of the program of the Laboratory of Psychology and Psychopathology (LPP) under the direction of Allan F. Mirsky. The permanent professional staff now consists of, in addition to Dr. Mirsky, Drs. Seymour S. Kety and Theodore P. Zahn. Senior Staff Fellows include Drs. Connie C. Duncan, Bruno J. Anthony and Loring J. Ingraham. Guest researchers include Drs. Frances H. Gabbay, Olive Quinn, Thomas Robinson, Katalin Vladar, and Mr. Lee Mann.

Two special experts are also part of our staff. Dr. Barbara P. Jones, a neuropsychologist, has been with us since 1986; Dr. Kathleen Jablonski, a systems programmer, joined the LPP this year. Finally, Dr. John Ingeholm, a biomedical engineer, was recruited to the LPP early this year, replacing the previous incumbent who resigned to accept a position in another institute.

While progress in the various projects has been satisfactory during the past year and a number of significant new findings have emerged which have been detailed below, it should be remarked that a significant part of the previous year's activity involved preparation for the review of the LPP in December 1987 by the Board of Scientific Counselors. Although it is our presumption that the review was favorable, the time devoted to preparation had a somewhat adverse effect on productivity.

Our major extramural collaborations are proceeding well. These involve the Prevention Intervention Research Center of the School of Hygiene and Public Health of Johns Hopkins University and the Oranim (Institute for Kibbutz Education) of the University of Haifa (Israel). New data from these collaborations have been incorporated in this summary and in the relevant individual project reports.

A brief outline of studies appears below.

A. Clinical Studies of Attention and Brain Function

1. Human Clinical Studies of Attention Disorder
2. Attention Disorders as Assessed by Event-Related Brain Potentials
3. Neuropsychological Evaluation of Psychiatric and Neurological Patients

B. Animal Models of Attention Disorders

1. Models in the Monkey of Generalized Seizures of the Absence Type
2. Brain Lesion and State Change Effects on Visual Attention

C. Autonomic Nervous System Activity in Attention and Psychopathology

1. Psychophysiological Responsivity and Behavior in Schizophrenia
2. Psychophysiological Concomitants of Minimal Brain Dysfunction in Children
3. Personality Factors and Psychophysiological Responses to Changing Stimulus Input

D. Interaction of Nature and Nurture in Disordered Behavior

1. Studies of Heredity and Environment in Schizophrenia
2. Studies on Etiological Factors in Schizophrenia
3. Genetic Factors in Response to Alcohol

A. Clinical Studies of Attention and Brain Function

1. Human Clinical Studies of Attention Disorder

We have been involved in a joint enterprise in which a variety of attentional, cognitive, autonomic, and electroencephalographically-derived tests are applied to a number of populations of experimental and control subjects. In some instances, a number of investigators are examining the same subjects. The populations have included epileptic persons, schizophrenic subjects, brain-lesioned subjects, dementing subjects, women with eating disorders, dyslexic men, and controls. The aim is to develop a profile of functioning for the several groups that will highlight the similarities and differences among them and will lead to a better neuropsychological characterization of their impairment, and provide insights into the pathophysiology of the non-lesion groups. In addition, attention measures are being applied to groups of normal and disordered pre-school and grade school children so as to be able to assess the prognostic value of early developmental signs of attention disturbances.

The major elements within this program include: the experimental paradigms of the Cognitive Psychophysiology Unit (Dr. Connie C. Duncan, head, Dr. Bruno J. Anthony, staff fellow, Dr. Frances H. Gabbay, guest researcher); the Neuropsychological Test Battery supervised by Dr. Jones (consultant) and Dr. Duncan; and the Autonomic Nervous System studies of Dr. Zahn.

The bulk of the neuropsychological examinations are conducted by Ms. Marie Elliott, with the participation of Drs. Jones and Mirsky. Dr. Duncan's Unit is applying techniques of cognitive psychophysiology to elicit and evaluate event-related potential components (auditory and visual) which have been shown to relate to attention, uncertainty and surprise (P300, N140, etc.). Similar experimental paradigms (e.g., the "odd-ball" method) have been applied to various groups of subjects with attentional disorders (eating disorders, schizophrenia, dyslexia, etc.) so as to be able to compare and contrast ERP component amplitudes, latencies and distributions. It is anticipated that these studies will help to illuminate (in conjunction with other methods) the nature of the similarities and differences among various attentional disorders. Dr. Zahn's work is reported below.

2. Attention Disorders as Assessed by Event-Related Brain Potentials

New ERP findings have emerged during the past year which contribute to the characterization of cognitive/attentive disturbances in patients with various types of neuropsychiatric disorders. In the Annual Report for 1986-1987, we highlighted the findings on patients with schizophrenia and seasonal affective disorders. It was found that schizophrenic patients show major differences in the visual but not the auditory P300 component of the ERP as a function of successful drug treatment. These data emphasized the possible roles of visual P300 as a state marker and auditory P300 as a trait marker in schizophrenia. A preliminary report of these findings has now been published.

Dr. Duncan has now submitted a report for publication describing ERP findings in seasonal affective disorders (SAD). Patients with SAD process visual (but not auditory) information more effectively if they respond to phototherapy. In a small number of patients studied intensively, it has been found that the visual P300 changes are incremental and can be monitored on a day-by-day basis. More details are found in the project description of Z01 MH00509-05 LPP.

A report is now in press describing the results of an ERP study of visual and auditory information processing in patients with absence epilepsy. The absence patients displayed markedly reduced P300 amplitudes to stimuli in both visual and auditory tasks. However, in one difficult auditory processing task (an auditory--tone discrimination--version of the AX form of the Continuous Performance Test or CPT), there were virtually no P300s elicited in the patients. This ERP difference was mirrored by major impairment in the patients' ability to execute correct responses in the auditory CPT--in both X and AX forms of the test. Thus, the normal subjects were able to execute both visual and auditory CPT task at the level of 90% correct or higher; in contrast, the patients' performance was quite variable, ranging from 90% correct on the X visual task to 65% correct on the AX auditory task. These data on absence epilepsy suggest a similarity to the findings with auditory information processing seen in schizophrenic patients described above: In both groups of subjects, there may be some overlap in the underlying pathophysiology which renders the processing of auditory stimuli more difficult than visual.

3. Neuropsychological Evaluation of Psychiatric and Neurological Patients

Our battery of neuropsychological tests provides a complete assessment of the executive, mnemonic, linguistic and attentive capacities of the human brain. The test battery has been administered in whole or in part to over 300 persons so far, and the information it provides will form the neurobehavioral database which will be used to interrelate, evaluate and integrate the various electrographic, biochemical and other physiological measures applied to the groups of patients with psychiatric and neurological disorders we study in the LPP. Two fruits of this neuropsychological effort have been published, or are in preparation for publication. One concerns a new analysis of the elements of attention, based upon factor analytic techniques applied to scores from our battery of attention tests. This analysis suggests that separate aspects of attention are assessed by different neuropsychological tests and can in turn

be related to separate regions of the central nervous system. This "elements of attention" model appears to have considerable utility in the study of neuropsychiatric disorders and may be of heuristic value in thinking about the process of attention, as well. Descriptions of the model have appeared in publications on the assessment of environmental toxins and on new clinical methods in neuropsychology. Parts of the attention battery were administered to school children in Baltimore in conjunction with the Prevention Research Center efforts. The other work concerns a neuropsychological profile of women with various types of eating disorders. There are a number of distinctive profiles associated with different subtypes; e.g., restrictors appear very different neuropsychologically from bulimics. Further, many of the cognitive differences from controls appear to be related to a core impairment in attention. Additional details can be found in the project description of Z01 MH 00509-05 LPP.

B. Animal Models of Attention Disorders

1. Models in the Monkey of Generalized Seizures of the Absence Type

Over the course of the last 15 years, staff now in the LPP have been interested in the development of techniques for simulating the generalized seizure patterns, as well as the behavioral accompaniments, of absence epilepsy. We have explored the use of electrical, chemical, and metabolic methods of inducing discharges. Most recently, we have done some experiments with compounds related to the metabolism of GABA (i.e., GBL, GHB). The parent compound GABA (gamma-amino-butyric acid) is thought to be a major neurochemical component of the inhibitory systems of the brain which control neuronal excitability. The work (now published) has suggested that these particular compounds produce effects which are more like sleep than like absence epilepsy. Nevertheless, such models permit the testing of various hypotheses about neurochemical events in absence epilepsy.

These studies were conducted under the direction of Visiting Scientist Dr. Michael Myslobodsky and with the participation of Drs. Richard Nakamura and Richard Coppola.

2. Brain Lesion and State Change Effects on Visual Attention

There is collaboration with LN and LCM in some parts of this work. One of the goals has been to specify the role of non-visual regions of the primate cerebrum in visual attention, and this work has used several techniques including lesions, stimulation, and electrographic recording of brain potentials under various lesion and non-lesion conditions. Other studies have been concerned with the recording and analysis of visually-evoked potentials under a variety of perceptual conditions, and with drug or sleep state effects on the mechanisms of visual attention.

One of the behavioral measures used in this study is the Generalized Attention Test (GAT) which is being developed at the NIMH by LPP staff. It represents an extension and modification of the Continuous Performance Test (CPT) of attention which was originally refined and perfected at the NIMH during the period 1954-1961. The CPT has been used extensively by various

groups of investigators, particularly in the study of epilepsy, schizophrenia, and metabolic illnesses. The GAT extends the concept of the CPT to involve a variety of cognitive dimensions (such as intra and inter-dimensional shifts), as well as parametric control of other perceptual variables. The GAT is designed for use with both humans and monkeys so that direct comparisons can be made of the results in each species. Preliminary results on monkeys indicates that they can be successfully trained to perform on this task and some of the data from the first monkey subjects are extremely promising. They indicate some unexpected allocations of function among sensory, motor, and "association" areas of the monkey cortex.

C. Autonomic Nervous System Activity in Attention and Psychopathology

This work is being carried out by Dr. Theodore Zahn.

The central focus of this research is the role of attentional processes and autonomic nervous system (ANS) functioning in psychopathology, especially schizophrenia. Studies are directed toward several basic issues: (1) the nature of the attention and ANS dysfunction, (2) the diagnostic specificity of the dysfunction, (3) state vs. trait issues, (4) the neurobiological basis of attention and ANS functioning.

We are continuing to collect data on our current schizophrenia protocol which is designed to study ANS activity during rest and task performance, compare ANS responses to stimuli differing in signal value with respect to frequency, amplitude, and habituation, and determine the nature of attention deficits in schizophrenia. When possible we test patients on standard neuroleptic medication as well as when drug-free because most non-NIMH investigators have only medicated patients available to them. This will provide information as to the extent to which their results are influenced by medication. Recruitment of subjects has been slow due to the slow turnover of Clinical Center patients, and the small number of patients available from other sources. We need to increase our sample of controls as well.

The diagnostic specificity of the findings is being addressed by comparing patients with affective disorder, panic disorder, obsessive-compulsive disorder (OCD), and autism with schizophrenic patients and normal controls. Over the years we have typically found that schizophrenics exhibit high levels of autonomic activity ("arousal"), slow adaptation and habituation, and attenuated ANS reactivity, especially to significant stimuli and situations. We have reported diagnostically specific patterns of ANS activity in both adults and children with OCD and in young men who were autistic children. These findings have been detailed in previous annual reports and have been published. We observed some differences between the adult and child adolescent OCD groups. We have finished testing a new sample of child/adolescent OCD patients and normal controls to see if this will replicate. We hope to be able to do a formal comparison of patients with affective disorder and panic disorder with schizophrenia in the coming year.

Our approach to the study of the biology of the peripheral autonomic measures includes studies of effects of pharmacological agents with more-or-less known neurobiological effects and by analyses of concurrent blood

samples for relevant biological activity. In one completed study, described last year, we tested the effects of yohimbine, an alpha-2 receptor agonist, in patients with panic disorder and controls. Yohimbine increased heart rate and subjective panic and anxiety more in the panic patients than in the controls, effects that were independent of alprazolam treatment. Recently completed analyses reveal that all groups showed about the same significant increase in plasma norepinephrine after the yohimbine challenge compared to placebo regardless of diagnosis or treatment. The greater effects on heart rate suggest greater beta receptor sensitivity in patients with panic disorder. However, the hypothesis that heart rate effects could be secondary to the greater subjective effects of yohimbine in the patients cannot be ruled out.

The psychophysiological and neuroendocrine effects of a "learned helplessness" procedure, designed to produce a temporary mood change similar to depression in normal subjects, have been studied in collaboration with NSB. Subjects are given an insoluble task of learning how to terminate bursts of loud noise which are presented periodically. This condition ("nonescape") is compared with an "escape" condition in which the same amount of noise is presented but each burst can be terminated by a learned response. Results on ten normal volunteers were presented last year and have been published. Higher electrodermal activity, higher plasma ACTH, a trend for higher cortisol, and a greater increase in dysphoric mood occurred after the nonescape compared to the escape condition. Preliminary analyses of the psychophysiological data on a larger (N = 19) group of controls and 14 unmedicated patients with affective disorder show significantly greater background electrodermal and cardiovascular activity in the nonescape vs the escape condition in both groups. This suggests that patients with affective disorder and normal controls have similar ANS reactions to a lack of control over aversive events. Patients who were symptomatic had significantly less EDA compared to controls and to a small group (N = 5) of euthymic bipolar patients independent of the situation suggesting that the low EDA frequently reported in depressed patients may be state related. An implication of these results is that the "learned helplessness" procedure produces a pattern of ANS activity that is clearly distinguishable from that occurring in patients with symptomatic affective disorders. Further analyses of these data are underway. Analyses of data for small groups of schizophrenic patients and women with premenstrual syndrome are also planned. In another study relevant to the neurobiological mechanisms underlying our ANS measures, we are testing the patients with focal head injuries, who are being recruited by LPP, on the ANS-attention battery we use with schizophrenics. This should help us determine if the deficits we see in schizophrenics could be due to localized brain dysfunction.

A study in collaboration with the CNG Branch on the offspring of parents with bipolar affective disorder is being written up for publication. The aim was to determine if some of the purported ANS markers for affective disorder, chiefly low electrodermal activity, could qualify as genetic markers. Our data show tnat low electrodermal activity is not a likely marker for affective disorder. On the contrary, the high-risk subjects showed electrodermal hyperactivity to the mild stress of task performance and relatively more activity from left-hand recording, suggesting these phenomena as possible genetic markers. Mood ratings showed markedly higher ratings of depression by

the high risk group just during a stressful task but no differences in anxiety ratings. Our data are consistent with a sensitization model proposed by Robert Post of the IRP in showing that persons at risk for affective disorder may exhibit depressed mood and/or exaggerated physiological activation during rather ordinary environmental stressors.

Another study designed to separate state and trait influences, a three-to-five-year followup of adolescents with diagnoses of obsessive compulsive disorder done with Dr. Rapoport's group, has been described in earlier reports. The most interesting results so far are that patients with high ANS arousal indices and slow habituation at baseline had the poorest outcome at followup suggesting that obsessive adolescents with a greater biological predisposition are more refractory to long-term remission than patients without this trait. We have tested a new sample of obsessive adolescents at baseline and also during clinical trials of two drugs. In another collaboration with CHP, children with diagnoses of attention deficit disorder and conduct disorder are being tested.

Dr. Robinson, a Guest Researcher in LPP, is continuing his studies on personality and psychophysiology in normal volunteers. During the past year the focus has been on a study of the relationship between personality variables and the dependent variables of simple, warned reaction time (RT) and the psychophysiological concomitants of RT performance. The major findings are that introverts, as measured by the Eysenck Personality Inventory had significantly slower RTs than extraverts, particularly at long foreperiods, and that they showed relatively less deceleration of heart rate under the latter conditions. These findings in introverts resemble our observations in schizophrenic samples over many years (although the patients show more pronounced deficits). The data from this study also confirms our previous impressions that Eysenck's "Psychoticism" scale is more aligned with a concept of psychopathy than with schizophrenia. Thus introversion may turn out to be a useful nonpathological model of at least some aspects of the schizophrenic process.

Finally, we are planning to test the children whom LPP is attempting to recruit from the Hopkins preventive intervention projects, who have have been screened for traits that may be predictive of future behavior problems. On the basis of previous research from our laboratory and elsewhere, we are optimistic that such traits as fearfulness, aggressiveness, and impulsivity may each be distinguishable from well-adjusted by our ANS and/or behavioral measures.

D. Interaction of Nature and Nurture in Disordered Behavior

1. Studies of Heredity and Environment in Schizophrenia

The project is composed of the following studies:

a. An intensive multidisciplinary study of a family with monozygous quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome.

The first study of this family was completed and published in book form in 1963. We have continued our contacts with this family to follow the clinical course of these women and to see how the course is related to earlier and current life experiences. A second intensive multidisciplinary study of these women was completed in June of 1981, and has been summarized in previous annual reports. As of the present time, one theoretical piece describing nature-nurture issues in the Genain quadruplets has been published and a second is in press in the Schizophrenia Bulletin. In this latter work, the status of the Genains is brought up to date, and a new theoretical view of the relation between early damage to the brain and later experiential factors in the Genains is propounded.

b. Studies of adoptees with schizophrenic parents and their biological and adoptive families.

This represents a completion of work done with a cohort of adoptees and controls that were obtained in Denmark in the 1960's. Although a considerable portion of this work has been published, much of the psychological information was not analyzed. A reanalysis of the original case material, which reaffirmed the original finding, has been published. A paper comparing disordered and non-disordered subjects in terms of reported stresses during development has been submitted for publication. The data suggest that intense familial stress during development can interact with a schizophrenic diathesis to produce more severe forms of disorder than occur under less familial stress.

c. A study of children of schizophrenic and control parents reared in town or kibbutz in Israel.

This study, begun in 1962, has involved a multidisciplinary (psychological, psychiatric, neurological) examination of a cohort of 50 children with schizophrenic parents (index cases) together with 50 matched controls. Half of each group was raised on a kibbutz, and half was raised with their nuclear families in cities and towns in Israel. The children were seen three times: once in 1966-67, when they were about 10-11 years of age, once in 1973 when they were about 17, and most recently in 1981 when they were in their mid-twenties. The results of the 1981 study suggest that being raised in a kibbutz environment is more likely to lead to major psychopathology, given a schizophrenic diathesis, than is growing up in the nuclear family within a city environment. A surprising finding was the large incidence of affective disorders, particularly in the kibbutz-index group. Another major followup of the Israeli cohort is now in progress, which will include brain CT scans, measures of psychiatric status, behavior on cognitive and attention tests, reanalysis of parental hospital records, reinterviews of the surviving parents, construction of family histories (to address the question of genetic vs. sporadic schizophrenia) and a survey of the status of the siblings of the index cases. At this time, approximately 80 of the original, surviving and traceable 97 subjects have been reinterviewed. (Thirty of the surviving parents have been seen, as well.) There is no suggestion of an increase in the number of study subjects that have developed schizophrenia since the 1981. However, there appears to have been an increase in the number with affective disorders, in both index and control cases; it may be that affective disorders will be found to be almost equally prevalent

among all groups in the study. However, the previous finding, that psychiatric disorder appears earliest in the kibbutz-index cases, obviously is still valid.

The objectives of all of these projects are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

2. Studies on Etiological Factors in Schizophrenia

Studies of the occurrence of mental illness in families have been useful in identifying familial forms of the illnesses and in the development of hypotheses regarding the form and strength of genetic and environmental factors in etiology. Where these major variables are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. A total national sample of 14,500 adult adoptees in Denmark, including 76 who have developed schizophrenia, provide the basis of one phase of this research; the other phase is represented by schizophrenic patients and their families residing in Roscommon County, Ireland, where the prevalence of schizophrenia appears to be three times higher than its prevalence in England and other Western countries. With respect to the Danish cohort, work has recently been completed on an analysis of the outcome of the "provincial" sample. This consists of a group of adopted-away offspring of schizophrenia parents raised in the region outside of Copenhagen. As in the case of the Copenhagen sample, relatives of index cases were found to have significantly more schizophrenia (latent or chronic) than relatives of controls. Further details of the recent progress in studies done under this protocol are contained in Z01 MH 02288-03 LPP.

3. Genetic Factors in Response to Alcohol

This project, which is being executed by Dr. Gabbay, seeks to evaluate the relative contributions of heredity and environmental factors in response to alcohol. Using a classic behavior genetics design, both mono- and dizygotic twins are administered standard doses of ethanol. The effects are assessed with a battery of behavioral and electrophysiological measures. Other investigations include surveys of drinking behavior and of the retest stability of alcohol effects.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1987 through September 30, 1988
Carmi Schooler, Ph.D., Acting Chief

Still limited somewhat by the small size of its full-time professional staff, the Laboratory of Socio-environmental Studies successfully continued its multi-pronged research program. One direction of research involves further analysis of previously collected survey data. Here we investigate how the different life circumstances of people in various positions in the social hierarchy explain differences in their psychological functioning. Other directions involve the development of several new lines of research aimed at explicating the scientific implications of the Laboratory's earlier findings on both normal and abnormal psychological functioning throughout the life course. These new approaches lead to a broadening of both the scientific techniques used and the topics investigated. Thus, the range of the Laboratory's research approaches has been expanded to include not only survey-based social psychological, sociological and cross-cultural research, but also initiatives in experimental cognitive psychology and psychopathology. In still other approaches, the Laboratory continues to develop and apply advanced statistical methodology and anthropological observation techniques to the study of the mentally ill and homeless in the community.

An important accomplishment this year in the Laboratory's continuing survey based investigation of the nature of social structural effects on psychological functioning is the completion by Schooler and Schoenbach of an examination of how some types of parental behavior may affect aspects of children's psychological functioning. The parental behaviors examined are control and support; the aspects of children's functioning examined are self-directed values and orientations, intellectual flexibility and distress. The latent variable linear structure equation analyses that were used permitted the purging of measurement error from the parents' and children's reports of parental behavior as well from the measures of the various psychological functions. The analyses controlled the effects, not only of relevant background variables, but also of the parents' psychological functioning. This latter control insures that the relationships found between aspects of children's functioning and parents' behaviors are actually due to parents' behaviors and are not artifacts of direct genetic transmission or of some environmental factor linked to both the psychological function and the parental behavior in question. Although reciprocal effects models could not be successfully estimated and despite the various anomalies and sex differences found, the results generally support the hypotheses. These hypotheses, derived from the Laboratory's research program and from the relevant literature, are that controlling parental behavior decreases children's self-directedness and intellectual flexibility and increases their distress, while supportive parental behavior has opposite effects. Most of the significant exceptions to this pattern seem to occur when parents exhibit non-stereotypic sex-role behavior that appears to have positive effects on their children.

Besides finishing their analyses of the effects of parents' behavior on American children, Schooler and Schoenbach have also continued their work on interview data of Japanese families. These interviews, which were gathered in collaboration with Japanese investigators in Tokyo and Osaka, are essentially replications of the American family interviews, and it is anticipated that they will be subjected to a similar course of analysis as were the data from the American families.

A second study completed in the Laboratory this year involving the analysis of survey data to examine the interrelationships between social structure and psychological functioning is that by Rosenberg, Schooler and Schoenbach centering on the causes and effects of self-esteem. Since the self-concept both causes and is caused by social factors, past research has sometimes treated self-esteem as a social force and at other times as a social product. What have generally been lacking in this large body of research have been investigations dealing with the reciprocal effects of the self-concept and various social and personal factors.

Rosenberg, Schooler and Schoenbach have attempted to deal with this issue through the use of latent variable linear structural equations analysis. Using this technique on the data from a sample of 1886 adolescent boys, they explored the possible reciprocal relationships between self-esteem and three important social and personal problems of youth: juvenile delinquency; poor school performance; and psychological depression. One of their most striking findings is the demonstration of how complex and varied the "mutual effects" of two variables may be. Each of the three bivariate relationships shows a distinctly different pattern of effects. Thus, they found that low self-esteem fosters delinquency while delinquency reciprocally raises self-esteem. The relationship between self-esteem and school performance turns out not to be reciprocal; school performance affects self-esteem but not vice versa. Finally, the causal relationship between self-esteem and depression proves to be reciprocal, with the stronger effect being from depression to low self-esteem. It is extremely difficult to see how such effects could have been predicted in advance. Such diversity must give pause to investigators who advance glib and simple explanations of the causes and effects of self-esteem and readily suggest ways in which self-esteem can be improved or even manipulated as a way of preventing social problems.

This year progress was also made in developing new experimental approaches to cognitive psychology. The aim has been to explain the psychological mechanisms underlying the Laboratory's earlier survey-based findings on the effects of socially structured environmental conditions on cognitive functioning. Leslie Caplan and Carmi Schooler completed an experiment designed to shed light on the psychological implications of earlier findings about the relationship of occupational self-direction and intellectual flexibility. Their hypotheses are framed in terms of a more general theory of complex environments directly linked to developments in cognitive psychology. During the experimental sessions, which lasted approximately five hours, 128 subjects learned a microcomputer drawing package under different conditions of training organization and practice complexity. During training, instructions were presented in either a random or organized order, and either with or without an analogical model

of the software package. Practice trials varied in both visual complexity and logical complexity. The results demonstrate that individual differences in background skills or experiences can affect how individuals begin to learn how to use a computer software package. They also suggest that as individuals gain experience in a new domain, differences in competence become less a function of background variables and more a function of the nature of the current experience. Finally, they demonstrate that the effects of experimenter-provided organization or analogical models during training depend on the complexity of the subsequent experience with the domain. In particular, models or other types of organization may help performance when one's experience in the domain has been complex, but hurt performance when the experience has been relatively simple.

In addition to completing this extensive experiment on learning processes, this year Caplan has also continued her research on the nature of the categories individuals use in their cognitive functioning. This problem is a basic one in the study of how people think. The two experiments on this topic that she completed this year provide further support for the important hypothesis about category representation that she has put forth together with Barr. This hypothesis proposes a model that distinguishes between two kinds of categories--intrinsic and extrinsic--and in doing so integrates and explains many otherwise puzzling findings in this area of research.

The Laboratory has also expanded its substantive and methodological interest in the study of psychopathology and social dysfunction in both community and hospital settings. The methodologies used to study these issues in the community this year ranged from advanced quantitative techniques developed and adapted within the Laboratory to qualitatively oriented anthropological observation. Thus, Schooler and Ronald Schoenberg continued their collaborative investigation with William Eaton of the Department of Mental Hygiene of Johns Hopkins University on the nature of schizophrenic symptoms in the representative community samples gathered by the Epidemiologic Catchment Area (E.C.A.) Program. Adapting recently developed techniques in Latent Class Structure Analyses, they have used this methodology on categorical symptom data to develop models of the components of schizophrenic symptomatology parallel to models that would be developed on metric data through linear structural equation analysis. Using such methods they have shown that what were apparently regional differences in symptom patterns are really results of regional differences in sexual and racial composition.

This year Liebow finished the data-gathering stage of his extensive anthropological observation study of homeless women in Montgomery County. He also completed the complicated task of coding and organizing his massive data files and has begun the process of developing a qualitative picture of the nature and state of the lives of women who find themselves homeless in the midst of comparative affluence.

The Laboratory this year also returned to the experimental study of psychopathology within mental hospital settings. With the helpful collaboration of the NIMH Neuropsychiatric Research Hospital at St.

Elizabeths, Schooler and Roberts have begun data collection in an experiment exploring the nature of the social dysfunction that seems to characterize schizophrenics. Earlier work in the Laboratory and elsewhere has consistently shown that not only are schizophrenics prone to avoid social interaction, but that they show a decrement in cognitive functioning as the intensity of social interaction increases. The reason for such social dysfunction remains unknown. It is plausible that the disruption that schizophrenics seem to experience in social situations may not result from the specifically human characteristics of the others. Instead, the generally complex nature of social situations may leave the schizophrenics in a state of information overload. The study begun this year tests this possibility by examining schizophrenics' functioning in an experiment in which the social and non-social conditions are equated in their degree of cognitive complexity. Using the computer's ability to both present stimuli and evaluate response accuracy the experiment evaluates schizophrenics' performance on a perceptual task comparing performance when accuracy feedback is presented by a person with performance when feedback is presented by a computer.

All in all, the various research approaches taken by the Laboratory this year would seem to provide the appropriate mix of continuity and innovation to insure that the Laboratory continues its past high level of achievement while increasing the range of its approaches to the investigation of the relationship of the social environment to the individual's psychological functioning.

Annual Report of the Laboratory of Cell Biology
National Institute of Mental Health
Michael J. Brownstein, M.D., Ph.D., Chief
October 1, 1987 - September 30, 1988

INTRODUCTION

A number of people have left the LCB or will leave in the near future: N. Buckley, C. Buckley, D. Mullen, A. Young, R. Lad, B. Conklin, A.-M. O'Carroll. We will miss them and wish them well.

Our summer employees--M. Warden, R. Zimmerman, B. McLaughlin, and D. Reddish have returned to school; we are grateful for their help. In addition to the above, several guest researchers have visited the Laboratory for short periods to learn techniques; more are expected next year.

Several new investigators have come to the Laboratory this year. J. Martin and B. Hoffman have joined Dr. Brownstein's group; D. Agoston, R. Lloyd, A.-H. Voltz, R. Fischer-Colbrie have come here to work with Dr. Eiden; H.-J. Wess and R. Lahti are visiting Dr. Bonner; R. Kanterman is studying with Dr. Axelrod; M.P. Padgett has moved from the NINCDS to the NIMH (Peptide Chemistry Unit) and K. Reynolds has transferred to the LCB from the NCI in order to establish, along with Heinz Arnheiter, a joint NIMH-NINCDS Transgenic Mouse Facility. Finally, two new and much needed secretaries have joined the LCB staff--S. Houser and S. Myers.

The LCB is made up of people with diverse skills and interests. Since their interests often overlap, they frequently collaborate with one another. They can be divided--somewhat arbitrarily--into three groups: neuroanatomists, biochemical pharmacologists/cell biologists, and molecular biologists. These groupings have more to do with special areas of technical expertise than differences in areas of scientific inquiry.

The most senior neuroanatomist in the LCB is M. Palkovits who continues to spend half of each year in Bethesda and half in Budapest. His colleagues, E. Mezey and S. Horváth, are presently visiting the Laboratory with him. In addition, C. Gerfen and W.S. Young have

independent neuroanatomical units and help bridge the gap between neuroanatomy and molecular biology.

J. Axelrod and M. Zatz continue to oversee strong programs in biochemical pharmacology; L. Eiden has contributed significantly to the development of an agent that may be useful for the prevention and/or treatment of AIDS infections at the same time that he has pursued his interest in neuropeptides; H. Okayama, T. Bonner, C. Weinberger, and M. Brownstein apply molecular biological methods to study development and intercellular communications.

Overview

Our goal is to understand the development and function of the nervous system. Our approach, in general, is a reductionist one. We want to discover molecular mechanisms that underlie specific biological phenomena such as neurotransmitter synthesis and release, neurotransmitter and hormone action, choice of cellular phenotype, etc. In particular, we are attempting to isolate, characterize, and study molecules with important roles in the brain and peripheral nervous system. Examples include neurotransmitter receptors, steroid hormone receptors, and factors that regulate all growth and differentiation. Information about such molecules is of practical importance. Cloned receptors can be used as tools for screening new drugs; the sequences of viral receptors can be used as a starting point for synthesizing antiviral compounds.

Cloning and sequencing of cDNAs and genomic DNAs

T. Bonner, A. Young, N. Buckley, and M. Brann have added a fifth subtype to their list of muscarinic receptors.

L. Matsuda and T. Bonner have isolated two cDNA clones that encode proteins structurally very similar to the known G-protein binding receptors. They are presently attempting to find the ligands that bind to these putative receptors.

B. Hoffman, having cloned a partial cDNA for the serotonin 5-HT1c receptor, has now isolated full-length clones encoding the 5-HT1c and 1a receptors. She hopes to isolate other 5-HT receptor cDNAs and to use these to study 5-HT pharmacology.

K. Koller, K. Kusano, and M. Brownstein have shown that mRNA extracted from AR42J cells encodes a cholecystokinin receptor. The receptor mRNA is about 3Kb in size. A

cDNA library prepared from the positive mRNA fraction is being used in an attempt to clone the receptor.

S. Lolait and his coworkers have isolated cDNAs encoding several α and β subunits of the GABA-A receptor.

A. Iacangelo and her colleagues have shown that pig chromogranin must be the precursor of pancreastatin.

J. Waschek has shown that the 5.2Kb of DNA upstream of the VIP coding region regulate tissue specific expression and induction of VIP mRNA in vitro. He is now preparing transgenic animals with a reporter gene appended to the regulatory sequences to test specificity of expression in vivo.

K. and N. Okazaki and H. Okayama have developed a highly efficient method for transfecting schzosaccharomyces pombe. This strain of yeast responds to many mammalian regulatory sequences, and mutants can have their defects complemented by mammalian genes. Thus, schzosaccharomyces pombe appears to be a very powerful cloning host for characterizing proteins involved in the cell cycle, DNA repair, etc.

Maribeth Eiden has isolated a cDNA that causes chemically-transformed Baby Hamster Kidney (BHK) cells to revert to a normal phenotype. She will now sequence this "recessive oncogene" (tumor suppressor) and attempt to determine its mode of action.

C. Weinberger has shown that lambda expression libraries can be screened for the presence of cDNAs that encode molecules such as the thyroid hormone receptor with radiolabeled ligands. He hopes to use this method to identify the dioxin receptor gene.

Dr. Brownstein has constructed a novel plasmid vector based on the original Okayama-Berg vector that should be useful for expression cloning in yeast, mammalian cells, and Xenopus oocytes.

Studies of receptors and second messengers

Drs. Bonner, Buckley, Brann, Felder, and Axelrod have shown that cells transfected with cDNAs encoding muscarinic receptors generate arachidonic acid when they are stimulated with acetylcholine. The various muscarinic receptors were found to differ in their ability to couple to G-proteins and second messenger systems (i.e., systems that increase arachidonic acid,

cAMP, and phosphatidylinositol turnover). Arachidonic acid release by m1, m3, and m5 receptor stimulation seems to result from activation of phospholipase A_2.

Dr. Bonner and his colleagues are examining genetically altered muscarinic receptors in order to determine the structural basis of their interactions with second messenger systems.

Drs. Burch, Axelrod, and Conklin have identified two different bradykinin receptors, B_1 and B_2, that can be distinguished on the basis of differential coupling to phospholipase A_2 and C.

Drs. Felder, José, and Axelrod have shown that dopamine DA-1 agonists can stimulate phospholipase C activity.

Drs. Burch and Axelrod have found that interleukin-1 (IL-1) stimulates the release of PGE_2 in Swiss 3T3 cells. Pretreatment of the cells with IL-1 enhanced PGE_2 release by bradykinin, bombesin, or thrombin. IL-1 increased phospholipase A_2, cyclooxygenase, and GTPase activities.

Dr. Jelsema has discovered that the GTP-binding protein, transducin, functions in receptor-mediated activation of phospholipases A_2 and C in bovine retina. Both of these enzymes are under inhibitary control. In addition, she has found that kinases modulate the action of G-proteins by phosphorylating them.

Drs. Mahan, Burch, and Kusano have looked for mRNAs in extracts of brain and intestine that encode receptors as a preclude to preparing and screening cDNA libraries.

Drs. Perrin and Muller have provided evidence that fodrin may be involved in the process of receptor recycling.

Anatomical studies

Dr. Mezey has used immunocytochemistry and in situ hybridization histochemistry (ISHH) to demonstrate phenylethanolamine-N-methyl transferase producing cells in the amygdala.

Drs. Horváth and Palkovits have shown by means of immunoelectron microscopy that somatostatin- and growth hormone releasing hormone-positive cells interact with one another. They have also begun to characterize the CNS lesion produced by N-methylamino-L-alanine in rodents. This neurotoxin is thought to be responsible for the Guamanian ALS-Parkinson's Disease-dementia complex.

Drs. Young and Lightman have continued to study the response of rat hypothalamic mRNAs to stress, opiate withdrawal, lactation, and hyperosmolality by means of ISHH. Drs. Young and Rance are using the latter technique to examine the normal anatomy of the human hypothalamus, and Dr. Young is studying the role of CRF in the inferior olivary nucleus in regulating eye movements in collaboration with Dr. Neal Barmack. In addition, he is working with Drs. Hadreen and Blackshear in studies of Huntington's Disease and regulation of ornithine decarboxylase, respectively.

Dr. Gerfen has demonstrated that the compartmental organization of the corticostriatal system is dependent on the laminar organization of the cerebral cortex. This suggests that a major function of the patch and matrix system is related to the manner in which inputs from the allo- and isocortical areas are integrated by the basal ganglia.

Dr. Gerfen has also examined the effects of nigrostriatal lesions and dopamine agonists or antagonists on second messenger systems in the striatum by means of ISHH. There were specific changes in mRNAs encoding G_s (but not G_i or G_o), protein kinase C (PKC1, 2, and 3) and phospholipase C (PLC I, II, and III). There were changes in dynorphin, enkephalin, and substance P mRNAs as well.

Studies of cellular growth, development, and transformation

Drs. Masuda and Okayama have generated a number of mutant NRK cells that are no longer sensitive to TGF-β or EGF. They hope to use these mutants to characaterize the components of the TGF-β transduction system and to understand the role of TGF-β in malignant transformation.

Expression of the α subunit of human chorionic gonadotropin in HeLa cells correlates well with tumorigenicity of these cells. The former is suppressed when transformed HeLa cells are fused with normal cells. Drs. Toyama and Okayama are constructing plasmids containing the HCG promoter upstream of a selectable marker. They plan to introduce these plasmids into transformed HeLa cells and then introduce a cDNA library from normal cells. Then by selecting against cells in which the HCG promoter is still active they hope to isolate novel recessive oncogenes.

Drs. Yamashita and Okayama have transfected v-Ki-ras-transformed NIH 3T3 cells with a human cDNA library. They are attempting to isolate colonies of cells that have reverted to normal (non-transformed) phenotype. They hope in this way to find human cDNAs encoding proteins with ras-suppressing activities.

Drs. Armbruster and Bertolotti are attempting to clone cDNAs from fibroblasts that extinguish the expression of liver-specific genes in hepatoma cells.

Chronobiology

Dr. Zatz continues to study the circadian rhythm in indole metabolism in chick pineal cells in vitro. Light has two effects on these cells: 1) to inhibit melatonin output and 2) to entrain the underlying pacemaker. Light and calcium influx appear to regulate melatonin production acutely via cyclic AMP, and the mechanism of phototransduction in the pineal differs in the pineal and retina. Current efforts are focused on understanding the mechanism of photoentrainment.

Mechanical, thermal, and optical signs of neuronal excitation

Measurement of tissue shrinkage/swelling and heat production--non-electrical signs of neuronal excitation and synaptc transmission--reveals important properties of excitable tissue which are not apparent when conventional electrophysiological methods are employed.

I. Tasaki and P. Byrne have greatly improved the sensitivity and time resolution of heat sensors. These devices can be used to analyze the process of energy metabolism in thin pieces of tissue. Piezoelectric transducers, on the other hand, can be used to detect small forces developed at the surface of nerve fibers during excitation. Four tissues have been studied: the garfish olfactory nerve, the guinea pig pituitary, the guinea pig superior cervical ganglion, and the bullfrog olfactory bulb. In general, excitation of cells (and axonal conduction) are accompanied initially by generation of heat and by tissue swelling. The former presumably results from cleavage of high energy bonds the latter from movement of water molecules into the tissue. Prolonged stimulation of tissues result in long-lasting shrinkage, the biochemical basis of which is being investigated. Tissue swelling seems to correlate with excitation; shrinkage seems to correlate with inhibition and hyperpolarization.

AIDS-Related research

M. Eiden and C. Wilson have succeeded in generating two gibbon ape leukemia and an HTLV1 recombinant viruses. These recombinant viruses are capable of infecting all cells infectable by their pathogenic wild type counterparts, but they cannot replicate in infected cells. Therefore, that can safely and conveniently be used to study interactions between viruses and their receptors and to look for drugs or antibodies that interfere with viral binding to cells. In addition, the recombinant viruses provide an efficient means of introducing cloned genes into infectable cells.

L. Eiden, P. Padgett, A.-H. Voltz, D. Rausch, and their collaborators B. Fraser, P. Nara, J. Liffson, N. Dunlop, and K. Hwang have continued to search for and characterize peptide analogs capable of inhibiting HIV infection and cytopathicity. They have reported that benzylated derivatives of CD4 (83-94) block infection in vitro by four HIV isolates with very different envelop gene sequences. They are searching for more potent compounds and preparing to test their candidates in SIV-infected monkeys.

Summary of Annual Report of Laboratory of Cerebral Metabolism
National Institute of Mental Health
October 1, 1987 through September 30, 1988

The Laboratory of Cerebral Metabolism continues to operate with two Sections. One, the Section on Developmental Neurochemistry, is located in Building 36; the other, the Section on Clinical Brain Imaging, is located in Building 10. Although there are overlapping interests and techniques used in their research, the spatial separation of the two Sections presents obstacles to the implementation of close collaborative efforts and intellectual interactions. This may possibly be an advantage because it ensures truly independent research programs for the two Sections.

Section on Developmental Neurochemistry
Louis Sokoloff, Chief

This Section is now coming out of a period of transition. During much of the last 3 years a major portion of the Section's time and effort was devoted to examining the allegations of a small group that the deoxyglucose method was invalid because of high levels of glucose-6-Pase activity in brain which caused significant loss of deoxyglucose-6-P from the tissue during the experimental period. In the development of the method, this possibility had been carefully considered, and experimental data had shown that, although there was a small amount of such activity, the level was so low that it had no detectible effects on the results within the first 45 minutes after a pulse of deoxyglucose. After 45 minutes the effects became detectable and became more significant with increasing time. The procedure was, therefore, limited to 45 minutes, after which correction could be made for those effects if it was absolutely necessary to extend the experimental period to longer times. The experiments in the Section by Dienel, Nelson, Cruz, Mori, et al. completed this last year have resolved the controversy. It was found that the original observations were correct; the claims of high glucose-6-Pase activity in brain were found to be based on methodological artifacts of the grossest kind. The last paper on these studies has recently been submitted to the Journal of Biological Chemistry.

As the studies on glucose-6-Pase were coming to fruition, the Section began to shift over to other projects that had previously been started or planned and interrupted by the studies on glucose-6-Pase activity. One was the refinement of the original model of the deoxyglucose method to incorporate new information about the intracellular compartmentation of the enzymatic activities on which the method is based. For the 45-minute experimental period used with autoradiography in animal studies, the old model was adequate, but with the long experimental periods required by PET scanners used for studies on man, it was necessary to incorporate consideration of the long-term fate of deoxyglucose-6-P. A complex 5-parameter model was developed, experimental data were collected in rats up to 120 minutes (by K. Mori, N. Cruz, et al.), and the equation of this new model was fitted by K. Schmidt to the data to obtain the best-fitting five rate constants. The new model takes into account the breakdown of deoxyglucose-6-P at late times and the recycling lack of the released deoxyglucose into the precursor pool. The new model produces considerable improvement in the accuracy

of the results obtained at long times beyond 45 minutes, but has no significant effect at shorter times. A manuscript on this work has been submitted to the J. Cerebral Blood Flow and Metabolism.

Work has been resumed on the development of a combined method to apply the deoxyglucose method to measure local cerebral glucose utilization simultaneously with the measurement of the local lumped constant with methylglucose. Under physiological conditions this is not necessary because the lumped constant is uniform throughout the brain and adequately stable. It can change, however, with pathological changes and can change locally with focal pathology. In such cases a method to determine the local lumped constant is necessary. K. Mori and most of the others in the Laboratory have done the necessary in vivo biochemical experiments to provide the data on which the theoretical basis of the method rests. It is now necessary to develop a suitable double label [^{3}H and ^{14}C] autoradiographic technique to measure both radioactive methylglucose and deoxyglucose simultaneously in the tissues by quantitative autoradiography. C. Smith, C. Kennedy, and H. Nakanishi are making progress in the pursuit of this goal.

E. Palombo, L. Porrino (Guest Researcher), and A. Tannenbaum have continued their studies in collaboration with I. Kopin and his colleagues in the NINCDS on the MPTP-primate model of Parkinsonism. They have applied the deoxyglucose method to studies of the acute effects of MPTP and found that it depresses glucose utilization almost everywhere in the brain, except in a few structures, most notably the substantia region pars compacta, which is the structure that is ultimately damaged by the drug that is responsible for the parkinsonian syndrome. This work has been published in Brain Research, and the results of studies on hemi-Parkinsonism are being prepared for publication.

C. Smith, N. Eng, and G. Deibler have completed their studies on the dilution of the precursor amino acid pool for protein synthesis with amino acid derived from protein breakdown in brain. This is essential information needed for the method for measurement of local rates of protein synthesis that they are developing. The results show that the flux of amino acids from protein breakdown is approximately 40% of the total amino acid flux into the precursor pool for protein synthesis. This is the first measurement of dilution of the precursor pool in brain thus far made, and a manuscript is being prepared for publication of this work in PNAS.

E. Kaufman, T. Nelson, and B. Driscoll are in the process of completing residual fragments left over from their previous projects while also initiating new projects in entirely new areas of research for them. All of these new projects are taking advantage of the opportunities afforded by Driscoll's establishment of neural cell cultures in the Laboratory. Driscoll is studying the possible influence of neurons derived from fetal rat brain regions that eventually would become targets of dopaminergic pathways on neurite development in neurons derived from regions that normally give rise to dopaminergic pathways when they are co-cultured together. The results indicate a positive effect, but Driscoll is still trying to determine its specificity. Kaufman is studying neuron-glia interrelationship in culture, and Nelson is studying intracellular compartmentation of substrates, enzymes, and carriers involved in transport

and/or metabolism in neurons and glia; both are in the process of developing, establishing, and testing methods needed to carry out their experiments.

M. Kies and G. Deibler are also in the process of switching from a project of many years in duration (e.g., myelin basic protein) to a new direction, the study of calcium-activated proteinases in the nervous system. These are enzyme activities that may have relevance to the degradation of peptide neurohumors and possibly to the turnover of membrane protein components. They, too, are in the process of developing and testing assay procedures needed for their studies.

Section on Clinical Brain Imaging
Robert Cohen, Chief

The major areas of effort in this Section have been 1) to develop new tracers or other approaches for the study of neurotransmitter function in normal and abnormal physiology; and 2) to apply available tracer methodologies to the study of neuropsychiatric disorders. To these ends the following achievements are notable.

[18F]-cyclofoxy, an opiate receptor dependent tracer, has been administered to man for the first time. Our initial PET (positron emission tomography) findings are that cyclofoxy accumulation is highest in the amygdala, thalamus, caudate and putamen but also apparent in the cingulate, anterior frontal cortex, and cerebellum. Compared to blood flow and glucose metabolic images, visualization of the amygdala is the unique feature of cyclofoxy studies. 6[18F]-DOPA presynaptic imaging of dopamine neurons has been achieved in rhesus monkeys. Preliminary findings suggest that the methodology is sensitive to the assessment of preclinical changes in this system. [125I]-BZM has been examined for utility as a post-synaptic dopamine receptor tracer for use in man with SPECT (single photon emission computerized tomography). Ex-vivo autoradiography studies in rats and mice, and postmortem autoradiography studies in monkeys have been promising. Moreover, we have had one successful SPECT study in a rhesus monkey.

We continue to find that PET measurements of glucose metabolism are increasing our understanding of the functional activities of the brain in psychiatric disorders. In schizophrenia, the data generated support functional abnormalities of the superior parietal and mid-prefrontal cortices, regions that appear by our PET studies to be important biological determinants of sustained attention in normals. Part of the functional abnormality appears to exist, only one of which appears to be sensitive to neuroleptic treatment. Furthermore, we observe a similar abnormality in manic-depressive disorder, but not in attention deficit, panic and obsessive compulsive disorders. In obsessive compulsive disorder we have observed an abnormally high metabolic rate in the orbital frontal cortex. These findings may suggest that the mid-prefrontal cortex abnormality reflects a vulnerability factor in the development of psychosis.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health
October 1, 1987 to September 30, 1988
Giulio L. Cantoni, M.D., Chief

In Fiscal 1988 the LGCB continued to develop its long term program of research without undue difficulties and with excellent scientific results. Like in previous years, the research program of the laboratory centers around projects that deal with the regulation of biological methylation reactions and their significance for various physiological processes. We are pursuing two principal lines of research: 1) in vitro our studies are designed to contribute to the basic enzymology and molecular structure of enzymes that synthesize or utilize S-adenosylmethionine and S-adenosylhomocysteine; 2) in vivo we investigate the importance of biological methylation in a variety of physiological and pathological processes such as cellular differentiation, macrophage chemotaxis and affective illness.

The research group of the LGCB is small and all the members of the staff work together as a team participating in one way or another in all the different projects since these are closely interrelated technically as well as conceptually. To augment the impact that our small group can make on a multifaceted research project we continue to favor collaboration with colleagues in Universities in this country and abroad. Especially noteworthy is the continue excellent collaboration with Prof. A. Razin and his colleagues at the Hadassah Medical School in Jerusalem, with Prof. M. Fujioka and his younger assistants in the Dept. of Biochemistry, Toyama Medical and Pharmaceutical University, in Japan, with Prof. S. Clarke at the Dept. of Biochemistry at UCLA, with Drs. G. Milligan of the U. of Glasgow and with L. Harvath of the DBBP of the FDA.

The objectives and principal results of the main projects under investigation are summarized below while technical details are described more fully in the individual project reports.

As the result of our work, as well as that of others it is now well established that in eukaryotes S-adenosylhomocysteine hydrolase (AdoHcyase) plays a critical role in modulating the intracellular AdoMet/AdoHcy ratio which is one of the principal factors that controls the utilization of AdoMet in various biochemical and biological systems.

It may be recalled that in eukaryotes AdoHcy is metabolized through a single metabolic pathway by AdoHcyase an enzyme first characterized at NIH in 1957 by de la Haba and Cantoni. AdoHcyase catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine.

S-Adenosylhomocysteine hydrolase has been purified from a variety of sources. Previous work has shown that the mammalian enzyme consists of structurally identical subunits. Our studies have been directed towards 1) the elucidation of the primary structure of the hydrolase by molecular cloning of its cDNA and by inference, its secondary and tertiary structure, 2) the determination of the specific polypeptide sequences that are involved in its binding, catalytic, and regulatory sites, 3) characterization of the conformational changes that accompany activation and binding of substrates and cofactors, and 4) crystallization of the

enzyme to provide an absolute three-dimensional structure by X-ray diffraction. AdoHcyase is an enzyme of unusual complexity: it always consists of a variable number of identical subunits (two for the plant enzyme, four for the mammalian enzyme and six for the bacterial enzyme) each of which binds tightly one mole of of NAD. The reaction catalyzed the enzyme consist in an oxidation-reduction cycle of enzyme bound NAD. We found that incubation with K^+, Mg^{++} and ATP leads to inactivation of the enzyme with loss of enzyme bound NAD. The inactive form of the enzyme binds cAMP, and can be reactivated upon incubation with NAD with displacement of cAMP. It seems therefore that NAD and cAMP bind to the same or similar sites on the enzyme.

The binding affinity of AdoHcyase from Dictyostelium discoideum for NAD is considerably smaller than that of the rat liver enzyme. It was of interest therefore to compare the amino acid sequence of the rat liver and the Dictyostelium enzymes in order to establish if the differences in NAD binding was reflected at the molecular level. Comparison of the amino acid sequence of the rat AdoHcy hydrolase (that has been established in this Laboratory) with that of the Dictyostelium enzyme indicated that the sequences are highly conserved. When the two sequences are aligned, 74% of the amino acids are identical, and if conservative changes are taken into account, the homology is 84%. The differences in amino acids between the rat and Dictyostelium enzymes appear to occur randomly throughout the sequence. The molecular basis for the difference in the NAD binding affinity is not immediately obvious since the putative NAD binding unit is practically identical in the two enzymes. It may be pointed out that Dictyostelium discoideum and rat are separated by one billion years in evolutionary terms. The striking homology in the amino acid sequences of ADoHcyase from these two species suggests that the conservation of the amino acid structure is required for the catalytic activity of the enzyme.

The cloned hydrolase was expressed in E. coli at an induced level reaching approximately 10% of the bacterial proteins. Site directed mutagenesis of the rat liver enzyme is being utilized to examine the structure/function relationship of different regions of the enzyme. The nucleotide sequence coding for rat liver AdoHcyase indicates that the enzyme is made up of at least two domains: a NAD binding domain, common to a large number of other NAD binding proteins, and a catalytic domain specific for this enzyme. Site directed mutagenesis will help delineate the nature and origin of these two domains. The cloned cDNA sequence is being utilized to examine the level of mRNA expression in different cell types and in various types of cells. Finally, we are examining the genomic organization of AdoHcyase in the rat.

Since AdoHcyase is the only enzyme known to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinylhomocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies on protein methylation have recently identified a novel class of protein methylation. We have identified a guanine nucleotide-dependent carboxyl methylation of several membrane proteins in mammalian cells. The methylation of membrane proteins of M_r 20-23K requires AdoMet, GTP or non-hydrolyzable GTP-analogs, and a cytoplasmic methyltransferase. The methylation was shown to be a carboxyl methylation by hydrolysis under basic conditions to produce methanol. However, the base lability of this methylation was much less than expected for aspartyl carboxyl methyl-esters. The role of guanine nucleotide-binding membrane proteins in regulating receptor mediated functions is well documented, and several families of these membrane proteins have been identified. The guanine nucleotide dependence and the physiologically reversible nature of carboxyl methylations suggests that these methylations may regulate the function of some guanine nucleotide-binding membrane proteins.

The capacity of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly. Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy.

1) In collaboration with Dr. A Razin, from the Dept. of Cellular Biochemistry of the Hadassah Medical School of the Hebrew University in Jerusalem, and several junior collaborators from the LGCB and The Hadassah Medical School, G.L. Cantoni has pursued further a project that was briefly described in earlier Annual Reports. It will be remembered that we have shown earlier that the transient hypomethylation observed in response to inducers of cellular differentiation in Friend erythroleukemia cells (MELC), (and probably in other differentiating systems), is due to a novel enzymatic mechanism which in the absence of DNA replication, brings about a modification in the pattern of DNA methylation by the specific removal of methyl cytidine (mC) residues and their replacements by cytosine. We have now found that treatment of MEL cells with 3-deaza-Ado and homocysteine during the first 20 hours after induction with HMBA or DMSO will completely inhibit the expression of the differentiated state (measured at 72-96 hours). By contrast when treatment with 3-deaza-Ado was delayed until 24 hours after induction, differentiation was not affected. The effect of 3-deaza-Ado was specific (neither adenosine nor deazaaristeromycin had any effect) and required the presence of homocysteine, a result that indicates conclusively that the effect is mediated by adenosylhomocysteinase. We have also established that the inhibition of differentiation produced by 3-DZA + Hcy is accompanied by the inhibition of hypomethylation induced by HMBA. Moreover, the inhibition of differentiation caused by 3-DZA + Hcy is strictly correlated with the length of the exposure to these compounds: if 3-DZA + Hcy are added together with the inducer but removed 8 hrs later there is no effect either on differentiation or on DNA hypomethylation; if the cells are exposed for 12 or 18 hrs after induction, differentiation is inhibited by 20 and 90%, respectively, and the hypomethylation is inhibited correspondingly. Exposure to 3-DZA + Hcy for the first 20 hrs after induction with HMBA results in complete inhibition of differentiation and the loss of methylcytidine is entirely prevented. We have also shown that if addition of 3-DZA + Hcy is delayed with respect to the addition of HMBA its effects are progressively diminished.

This transient decrease in 5-methylcytidine that is induced by HMBA is not affected by exposure of the cells to protein synthesis inhibitors such as cycloheximide, whereas differentiation is substantially inhibited under these conditions. The striking correspondence in the timing of the inhibition of differentiation and of the HMBA induced DNA hypomethylation produced by 3-DZA + Hcy adds weight to the hypothesis that this limited and specific modification of DNA structure is necessary but probably not sufficient for the expression of the differentiated genotype.

2) Macrophage chemotaxis: Studies on the inhibition of RAW264 macrophage cell line chemotaxis by 3-deazaadenosine led us to postulate that incubation of cells with 3-deazaaadenosine inhibits methylation reaction(s) required for the formation of functional mRNA coding for one or more chemotaxis proteins. Efforts to identify proteins that may play a central role in RAW264 chemotaxis have been limited because chemically defined attractants for RAW264 cells have not been available. This problem has been overcome by the isolation of a stable cell hybrid from a fusion between human leukocytes and a thioguanine-resistant RAW264 cell line. The hybrid expressed functional genes for chemotaxis to fMet-leu-phe, a commercially available synthetic attractant. Binding of fMet-leu-phe to hybrid cell membranes indicated that the binding constant was 2 nM and each cell had an average of 1200 receptors. Oxidized fMet-leu-phe was an attractant for the cell hybrid, granulocytes from several species (mouse, guinea pig,and rabbit), and human monocytes. However, human neutrophils do not migrate to oxidized fMet-leu-phe. These observations indicated that the human neutrophil may be unique in its lack of chemotactic responsiveness to oxidized fMet-leu-phe, and suggested that the fMet-leu-phe receptor complex or chemotaxis transduction mechanism may be different in human neutrophils than in other phagocytic leukocytes. We have shown that one or more guanine nucleotide binding proteins are required for chemotaxis by RAW264 and the hybrid cells. This conclusion is based on the observation that chemotaxis of either RAW264 or hybrid cells is inhibited upon incubation of the cells with either cholera toxin or pertussis toxin. For both toxins, entry of the toxin into the cell is required and there is a correlation between toxin-catalyzed ADP-ribosylation of a guanine nucleotide binding protein and the inhibition of chemotaxis. Although both cholera toxin and pertussis toxin affect cAMP levels, elevated cAMP levels per se do not inhibit chemotaxis. By immunochemical and electrophoretic techniques, the pertussis toxin substrate involved in chemotaxis has been identified as G_i-2, a protein that was also found in brain.

It is likely that G_i-2 is the guanine nucleotide binding protein that couples chemotactic receptors to an effector protein such a phospholipase C or ion channels. A second family of guanine nucleotide binding proteins that are not substrates for either cholera toxin or pertussis toxin has been identified in RAW264 cells. These membrane proteins were identified by radiolabeling the proteins with [methyl-^{3}]AdoMet in the presence of GTP. The properties of these proteins and the details of their identification are presented in more detail in the report for project Z01 MH 00931-15 LGCB. The RAW264 system will be used to test the role these proteins may have in chemotaxis.

In the Fall of 1987 and in May 1988, Prof. Razin returned to NIH as a guest of the LGCB where he discussed the collaborative research detailed above and plans for further experimental approaches. In February 1988 Prof. V. Andreoli visited the LGCB to collaborate with Drs. Cantoni and Mudd in the preparation of a manuscript on the relationship between biological methylation and affective disorders.

Late in October 1987, Dr. Cantoni and Dr. A. Razin with the support of FEBS and NIMH convened an Advanced Course on Cellular Differentiation and DNA Methylation in Faro, Portugal. Twenty-five distinguished lecturers from France, Germany, England, Israel, Japan and USA participated as invited speakers and 73 colleagues, many of them under the age of 30, attended the meeting which was very successful. In November 1987, Dr. Cantoni was invited as Visiting Professor at the College de France in Paris where he delivered a series of four lectures on Biological Methylation. The College de France is one of France's oldest and most distinguished institutions of higher learning having been founded in 1525 by Francois I. Dr. Cantoni also lectured at the Biozentrum, in Basel, at the U. of Strasbourg, and at the Institut Jacques Monod in Paris. Dr. Aksamit attended the 24th National Meeting of the Reticuloendothelial Society in Kauai, Hawaii; Dr. Backlund attended a Phagocyte Workshop and the Annual Meeting of the American Federation for Clinical Research in San Diego, CA; Dr. Kasir attended the meeting on Genome Mapping and Sequencing in Cold Spring Harbor, New York.

Annual Report of the Laboratory of Molecular Biology
National Institute of Mental Health
October 1, 1987 - September 30, 1988

Werner A. Klee., Ph.D., Chief

Introduction

An unique feature of the Laboratory of Molecular Biology is the fact that it has, since its inception in 1984, operated with a system of rotation of the Chief among the three Section Chiefs. The system works well, at least in part because of the good will and mutual respect of all involved. In the past year, as in previous years, the laboratory has prospered and functioned as a coherent, interactive unit. Each section made significant progress toward its research goals and each has contributed in important ways to the scientific work of the other groups within the laboratory. Each section continues to be a small operation consisting of a chief, one or two technicians, and several post-doctoral fellows. The small size promotes direct scientific contact between senior and junior staff members and encourages scientific exchanges both within the laboratory and with colleagues elsewhere. The work of the laboratory is highly regarded in the scientific community. We are all very proud of the fact that Howard Nash has recently been elected to the American Academy of Arts and Sciences.

The current progress of the individual sections is outlined in detail in the summaries of research that are presented below. The Section on Biophysical Chemistry, headed by Dr. David M. Neville, Jr., has further elucidated the way in which proteins, epitomized by protein toxins, are transported across cell membranes and intracellular compartments. Knowledge about the toxins was used to construct potent and selective immunotoxin reagents with cleavable cross-linkers which are now being studied in animals. The Section on Molecular Genetics, headed by Dr. Howard A. Nash, has shed important new light on the mechanism of genetic recombination, the process by which cells alter the information content of DNA. The group has also isolated several mutants of Drosophila that respond abnormally to halothane and, in different ways, to enflurane anesthesia. The Section on Regulatory Proteins, headed by Dr. Werner A. Klee, has characterized the the signal transmitting domain of G-protein coupled receptors using fragments identified by antibody screening. In related studies, the group has developed a general method for inactivating GTP-binding regulatory proteins in natural membranes which are then competent in reconstitution experiments.

SECTION ON BIOPHYSICAL CHEMISTRY

David M. Neville, Jr., M.D., Chief

Protein Translocation Across Membranes

Most macromolecular components entering (or leaving) cells are routed through vesicles and never cross the insulating membrane barrier. Certain molecules however do make this transition and in a highly specific manner. Examples are transmembrane proteins which can function as devices to transmit signals across the surface membrane or transmembrane protein channels which transport and thereby regulate flow of ions, electric charges and neutral molecules. The insertion or retrieval of the transmembrane proteins from the plasma membrane to intracellular membrane stores profoundly affects the properties of cells. Yet little is known about the chemical nature of these events. Plant and bacterial protein toxins are another group of proteins which are translocated

across membranes and in some cases form ion conducting channels. These proteins report their presence in the cytosol compartment by their toxic enzymatic activities or channel forming properties. Because of this the insertion and translocation process can be monitored, and the protein toxins serve as useful models in the study of membrane translocation events.

The study of toxin transport processes and toxin substrates asks several profound questions.

1) How does a highly charged hydrophilic macromolecule cross the lipid bilayer?

2) What controls the high degree of cellular specificity exhibited by these translocation processes?

3) How do the upstream events of toxin processing and routing influence the translocation step.

Over the past three years Dr. Thomas Hudson in our Section has performed studies which shed considerable light on how diphtheria toxin crosses the membrane. Most importantly, the translocation step has been identified as the rate limiting step in the intoxication process (once the initial lag period has ended) for diphtheria toxin, pseudomonas exotoxin A, ricin and modeccin. This means that by monitoring a latter step such as protein synthesis inhibition the translocation step is monitored. This permits a quantitation of which toxin domains and resides within these domains promote translocation. To eliminate toxic binding as a variable toxin domains or domains carrying point mutations are linked to the same monoclonal antibody and assayed on target cells binding the antibody with higher affinity than toxin. When this analysis is applied to diphtheria toxin the 17 kd amino terminus is found to enhance translocation 100 fold. However whole animal toxicity is also increased 100 fold even in B chain point mutants which have 1/1000 of the wild-type binding activity. Since we have determined the energy sources for DT translocation to be either trans plasma membrane voltage gradient or proton gradient it may be possible to distinguish alternate routing and translocation paths for these mutants. Possible interrelationship between binding and translocation domains requires elucidation.

Synthesis of Crosslinking Reagents which can be Cleaved within the Endosome Compartment for *in vivo* Drug Targeting Therapies.

If immunotoxins are to reach their therapeutic promise as effective *in vivo* cell type specific toxic reagents, then ways must be found to increase the current therapeutic margin. This may be accomplished by either increasing the killing efficacy of the immunotoxin to that of the parent toxin or decreasing the toxicity toward non-targeted cells by some means. Current efforts have largely focused on inclusion of the toxin translocation function located in the B chain while eliminating from the B chain those regions responsible for the non-specific toxicity, either by deletion of regions or by point mutation. Dr. Srinivasachar in our section is developing a different approach based on the use of a new class of acid cleavable crosslinking reagents. We propose that these agents can be used to deposit in the endosome a toxin or other protein in an uncrosslinked state which was transported into the cell as a crosslinked protein. Thus toxins can be freed from the steric constraints of conjugated antibody. In addition toxin domains leading to non-specific toxicity which operate in the extracellular compartment can be blocked extracellularly but unblocked within the endosome if this function is required for either the productive routing or the membrane translocation step. Anti-CD5 immunotoxins based on diphtheria toxin and ricin and constructed with cleavable crosslinkers have markedly increased efficacy. In addition reversibly blockade of toxin binding domains is possible, reducing non-specific toxicity. These findings are now being extended to animal studies.

The Design of Immunotoxins for the Treatment of Autoimmune Diseases

It is generally believed that multiple sclerosis, lupus erythematosus and early onset diabetes are autoimmune diseases. Increasingly, attention is being focused on provocative reports which indicate that certain subsets of mental illnesses may also have an autoimmune component to their etiology. Autoimmune diseases occur when the immune system's defense mechanisms are turned against a person's own body or parts of the body. The initiating causes of these diseases are poorly understood but both environmental factors and genetic factors seem to be involved.

Current therapy for autoimmune diseases aims at inhibiting the part of the immune system causing the damage. Our Section is working to develop very highly selective drugs to inhibit parts of the immune system such as suppressor T cells or helper T cells (T-4 lymphocytes). To do this, we use a totally new class of drug: immunotoxins. Immunotoxins consist of a powerful toxin linked to a monoclonal antibody which can seek out and bind to only certain types of cells. The toxins which come from poisonous plants (ricin) or harmful bacteria (diphtheria toxin) are extremely potent and have specific machinery for entering and killing cells.

The goal of our program is to understand the toxin entry machinery sufficiently well so that we can make therapeutically useful selective reagents. To this end Dr. Jon Marsh, working in our Section has developed a murine model system in which the effects of immunotoxins (administered *in vivo*) on immune system cell number and type may be observed. The model system is yielding information on (1) immunotoxin mediated efficiency of cellular killing between subsets, (2) repopulation of subsets from precursor pools, (3) compartmentalization of subsets from intraperitoneal and intravenous administered immunotoxins.

SECTION ON MOLECULAR GENETICS

Howard A. Nash, M.D., Ph.D., Chief

The Process of Lysogeny

This project is concerned with understanding the mechanism of one of the few ways that cells can alter the information content of DNA: genetic recombination. We want to know how a cell manages to align two DNA sequences and then accomplish the transfer of genetic information from one to another. We have taken a biochemical approach to this problem and have studied one particular genome rearrangement - the integration of DNA of the bacterial virus lambda into the chromosome of its host, *E. coli*. In the past we have made substantial progress in determining the overall features of this reaction and the biochemical principles that underlie some of its individual steps. Our current work seeks to answer questions about the order in which DNA strands are exchanged, the rates for various steps in the pathway, and the way in which an accessory protein assists the recombinase in initiating the reaction.

Our earlier work had shown that the breakage and rejoining of DNA during lambda integrative recombination takes place in two steps. First, one strand from each parent is broken, exchanged, and rejoined; this creates an intermediate called a Holliday structure. Subsequently, the Holliday structure is resolved into a completed recombinant by breakage, exchange, and rejoining of a second strand from each parent. We have now determined that there is a fixed order to the two sets of strand exchanges. Using two different experimental strategies we find that exchange occurs on the left of the the crossover region before it occurs on the right. We have also determined the features of the recombination locus (attachment site) that govern this asymmetry. Our data rule out the participation of local DNA sequences within the crossover region and show that the asymmetry is fostered by protein binding sites that lie 50 to 150 base pairs away from it, in the so-called arms of the attachment site. In our most decisive experiment, we have switched the position of these

arms with respect to the exchange region and found that the bias in strand exchange is reversed. This artifice not only proves that the bias in strand exchange reflects global rather than local features of the recombination locus, it permits us to test the relationship between DNA sequence matching and strand exchange. We find that the position of Holliday structure formation dictates the location of essential homology, proving that the two are casually related.

Kinetic studies can reveal what step(s) limit the rate of a biochemical reaction. We have mutated a kinetic study by determining the dependence of the rate of *in vitro* recombination on the concentration of one attachment site. For this study we focused on *att*B, the attachment site of the *E. coli* host, because our earlier work showed that it functions as a passive partner in the reaction, being captured by a nucleoprotein array assembled at the viral attachment site, *att*P. We find that the rate of recombination is proportional to *att*B concentration over a wide range, including values as high as 500 nM. This implies that *att*P is not saturated with *att*B even though *att*P concentration is fixed in these experiments at only 6 nM. The failure to saturate could come about in two ways: when *att*P and *att*B come together to make a synaptic complex, either recombinant products arise at a very rapid rate or synaptic complexes have a great propensity to disassemble before strand exchange can occur. To distinguish these possibilities, we have examined the kinetics of recombination under conditions where the rate of conversion of synaptic complexes to products is artificially reduced; under these conditions, *att*B still fails to saturate *att*P. We conclude that integrative recombination is a low affinity reaction in which the partner sites have, at best, a poor tendency to interact with on another.

Integrative recombination is carried out by two proteins - a virally encoded recombinase, Int, and an accessory protein encoded by the host, IHF. IHF is a sequence-specific DNA binding protein that this laboratory discovered because of its role in recombination. Genetic and biochemical studies have made it clear that IHF is a ubiquitous accessory protein in *E. coli*, playing important roles in processes as diverse as the packaging of viral DNA, the initiation of replication of plasmids, the transposition of diverse genetic elements, and the control of gene expression. Our previous studies of the role of IHF in recombination suggested that this protein helped to arrange *att*P into an ordered nucleoprotein array. One way in which IHF might do this would be to bend DNA so as to expedite the folding of *att*P into a compact form in which Int protomers could interact with one another. We have now tested the capacity of IHF to bend DNA. Our results show that the naked DNA is straight but that IHF introduces an easily detectable bend.

Genetic Neurobiology of Drosophila

Although the response to general anesthetics can be rather specific - loss of pain sensation and consciousness but retention of autonomic functions and simple reflexes - it has been hard to localize the action of anesthetics at either the anatomic, cellular, or molecular level. We are in the process of isolating and characterizing mutants with altered response to a general anesthetic in the expectation that some of these mutants will help highlight the target(s) of anesthetic action. We have previously established a screening procedure for determining in a semi-quantitative way the response of populations of fruit flies to the general anesthetic halothane. We have applied this procedure in order to select individuals with an abnormal response from amongst a population of mutagenized flies. While most candidate flies with unusual responses proved to be false positives, we have succeeded in identifying four resistant and two sensitive lines from over 20,000 mutagenized offspring. Initial characterization showed that these lines differed in their response to halothane but were distinguished from each other in their response to a second anesthetic, enflurane.

Our current efforts have been focused on characterizing these mutants first from a genetic and then from a pharmacological point of view. Unfortunately, the sensitive lines rapidly acquired genetic modifiers that reduced the anesthesia response phenotype to barely detectable levels. The resistant lines have proven more stable, although two of them show low viability. To map these mutations,

we have developed a simpler screening procedures for the anesthetic response that do not depend upon large numbers of flies. The assay reproducibly distinguishes mutant from control stocks of flies and thus opens the way to assess the linkage of the anesthetic response mutation to easily scored genetic markers whose chromosomal location are known.

SECTION ON REGULATORY PROTEINS

Werner A. Klee, Ph.D., Chief

The Section on Regulatory Proteins is engaged in a program aimed at understanding the mechanism of signal transduction across cell membranes. Receptors on the cell surface, such as those for the opiates, are coupled to enzymes, such as adenylate cyclase, on the inside of the membrane via the mediation of GTP-binding regulatory proteins (G-proteins). In this manner, information is transmitted to a cell from neighboring cells and from the fluid environment. The goal of the work is to dissect this system into its parts, and study each of the components of the system both in isolation and as a reconstituted functional entity.

All receptors share two essential properties: they bind ligands, and transmit the information of whether or not an activating ligand (agonist) is bound. Many receptors send information to one of several GTP-binding regulatory proteins (G-proteins) which have very similar amino acid sequences. This family of G-proteins includes at least 4 types of Gi, Go, and transducin. (We consider that Gs, the G-protein which activates adenylate cyclase is sufficiently different from all the others in structure that it forms a separate class.) Receptors coupled to the Gi class of proteins include, among others, opiate, muscarinic and bradykinin receptors and the photon (or more properly, perhaps, retinal) receptor, rhodopsin. Activation of G-proteins by agonist occupancy of these receptors ultimately results in activation, or inhibition, of one of several enzymes including phospholipase C, cyclic GMP phosphodiesterase and adenylate cyclase. We reasoned that receptors of this class, which must all interact with very similar regulatory proteins, might share structural features responsible for these interactions.

The availability of a battery of monoclonal antibodies directed against defined regions of rhodopsin (developed by Dr. Paul Hargrave and his collaborators at the University of Florida), allowed an experimental test of the hypothesis. For this test we used opiate receptors from NG108-15 neuroblastoma X glioma hybrid cells specifically substituted with the synthetic opiate [^{3}H]-3-methylfentanylisothiocyanate (superFIT). We found that one of the 46 anti- rhodopsin monoclonal antibodies tested also recognizes opiate receptors. The epitope against which this antibody is directed corresponds to the fourth cytoplasmic segment of the rhodopsin molecule immediately following the seventh (putative) transmembrane helix. A peptide corresponding to this region, rhodopsin 310-321, blocks interaction of the antibody with both rhodopsin and opiate receptors. An amidated peptide corresponding to the homologous region of the porcine brain muscarinic receptor, residues 422-431 (N10L) also blocks the antibody. Perhaps surprisingly, the peptide stimulates the GTPase activity of a number of purified G-proteins, but to somewhat different extents depending on the protein. The G proteins studied to date include Gi-1, Gi-2 and Go purified from bovine brain and transducin from bovine retina. As might be expected, the muscarinic receptor derived peptide is more effective as a simulator of the brain G-proteins than it is of transducin. Conversely, a homologous peptide corresponding to the photon receptor, rhodopsin, is much more effective as an activator of the GTPase activity of transducin than of the brain G-proteins. These experiments suggest that N10L and the homologous peptides of other receptors correspond to an important part of the receptor domain directly responsible for information transmission and show that it can work in a completely defined system.

Many experiments can now be performed using only purified proteins and peptides that should greatly clarify the mechanism of receptor action. As more receptor sequences become available much will be learned using this approach about receptor specificity and selectivity. We have found, for example, that other peptides, which display sequence similarities with portions of the third cytoplasmic loop of the G-protein coupled receptors, can also activate some of the G-proteins. These experiments suggest that the conformational change responsible for receptor activation as a result of agonist occupancy may consist of the assembly of a structure consisting of these parts of the third and fourth cytoplasmic domains.

Among the important unsolved problems related to trans-membrane signalling is the question of which G-protein is actually coupled to which receptor in the cell. Although reconstitution experiments with purified components in lipid vesicles are possible, and have been done, the results are disappointing in that it is clear that almost any G-protein can be induced to couple with almost any receptor in these unphysiological circumstances. We reasoned that reconstitution experiments performed with the actual cell membranes, in as nearly native state as possible, have a much better chance of demonstrating the specificity of receptor-G-protein interactions which must exist in the cell. We had previously shown that such reconstitutions are readily effected if the G-proteins were first inactivated *in-vivo* with pertussis toxin. However, some G-proteins, particularly many coupled to phospholipase C activation, are not inactivated by pertussis toxin treatment. A good example is the G-protein which couples bradykinin receptors to phospholipase C in NG108-15 cells. We have therefore sought to develop a general method to inactivate any G-protein without harming the membranes in which they function. The alkylating GTP analogue fluorosulfonylbenzoylguanosine (FSBG) was chosen as a likely reagent. This compound is very similar to GTP in shape and polarity and would be expected to bind at the GTP site of G-proteins. It also contains an alkylating group capable of forming covalent bonds with appropriately positioned nucleophiles on the protein. Treatment of NG108-15 membranes with FSBG results in the loss of bradykinin stimulation of phosphatidylinositide breakdown, as anticipated. Basal activity is largely unaffected and the enzyme can still be fully activated by calcium ions. Thus, FSBG treatment has not affected phospholipase activity directly. Furthermore, the number of bradykinin receptors is unchanged although these are of low affinity, as they would normally be in the presence of GTP. Final proof that FSBG treatment has inactivated the G-proteins was achieved by the demonstration that addition of a mixture of G-proteins purified from bovine brain restores the ability of bradykinin to stimulate phosphatidylinositol breakdown in FSBG treated membranes. Thus, use of this reagent should allow a more physiologically relevant rest of the specificity of bradykinin and other receptors for individual G-proteins.

ANNUAL REPORT OF THE LABORATORY OF NEUROCHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
OCTOBER 1, 1987 THROUGH SEPTEMBER 30, 1988

During the last year, we have continued our basic and clinical studies of the tetrahydrobiopterin-dependent aromatic amino acid hydroxylating systems.

Studies of the mechanism of the reaction catalyzed by pure rat liver phenylalanine hydroxylase have provided insights into the interaction of the regulatory and catalytic domains of this enzyme. Our results indicate that the division of function between these two domains may not be as sharp as has been assumed. Thus, through studies of the phenylalanine hydroxylase-catalyzed reaction under conditions where the hydroxylation reaction is completely "uncoupled" from oxidation of the pterin cofactor, conditions where the enzyme is activated by lysolecithin and tyrosine serves as a substrate analogue, it has been shown that there is not even a transient hydroxylation of the tyrosine. Nonetheless, this amino acid, which we have shown occupies the catalytic site, plays the role of a regulator or facilitator of the hydroxylase catalyzed reaction.

Another aspect of the phenylalanine hydroxylase reaction was probed with the use of a different analogue of phenylalanine, namely, p-methyl-phenylalanine. We demonstrated that pure hepatic phenylalanine hydroxylase catalyzes the hydroxylation of this compound predominantly on the methyl group rather than on the benzene ring; molecular oxygen is the sole source of the oxygen in the hydroxylated products. These results implicate a ferryl ion species (FeO^{2+}) as the active hydroxylating agent in phenylalanine hydroxylase-catalyzed reactions. This is an important insight into the mechanism of action of phenylalanine hydroxylase and the role of the essential protein-bound iron in the hydroxylation reaction.

We have found that phenylalanine hydroxylase in hepatoma cells is in a much higher state of activation than the enzyme in normal liver cells. The properties of the enzyme in hepatoma cells are consistent with the possibility that the enzyme is more highly phosphorylated. These findings raise the possibility that phenylalanine hydroxylase in tumor cells is regulated in a different manner compared to its mode of regulation in normal cells.

We have continued our studies of the molecular biology of the pterin-dependent hydroxylases and the ancillary enzymes. Dihydropteridine reductase from human liver was cloned; the nucleotide sequence was determined and the complete amino acid sequence was deduced. The gene has been expressed in monkey cells and, more recently, in E. coli cells. In the latter system, the expressed enzyme level approaches 1% of the crude E. coli protein. The gene has been localized to human chromosome 4, close to the site of the lesion in Huntington's disease, but sufficiently distant from this site to exclude the involvement of the reductase in this disease. Interestingly, the reductase is not close to any of the hydroxylases, which are located on chromosomes 11 and 12.

We have purified and prepared specific antibodies to one of the enzymes involved in the de novo synthesis of BH_4, 6-pyruvoyltetrahydropterin reductase. Through the use of the antibody, we have gathered additional evidence supporting the role of this enzyme in BH_4 synthesis in brain tissue.

Follow-up studies of the tetrahydrobiopterin (BH_4) deficit in the CSF of patients with Alzheimer's disease (AD) have led to the important finding that it is only the subgroup of these patients with movement disorders who have low CSF BH_4 levels. This finding identifies the group of AD patients who are the most suitable candidates for a planned intervention study in which BH_4 will be tested for possible beneficial effects.

One of the long-term goals of our research, i.e., the search for possible new roles for BH_4, was advanced during the last year with the finding that BH_4 may play a role in hematopoiesis. It has been known for sometime that the human red cell contains all of the enzymes needed for BH_4 synthesis. The reason why these cells can synthesize BH_4, however, has remained obscure. Using mouse erythroleukemia cell as a model system, we have found that BH_4 synthesis is necessary for the proliferation of these cells. On the one hand, compounds which cause these cells to differentiate and produce hemoglobin also shut down BH_4 synthesis. On the other hand, specific inhibitors of BH_4 synthesis inhibit DNA synthesis but do not cause differentiation. Although the site of action of BH_4 in these cells remains to be elucidated, our findings suggest that the hematopotesis status of BH_4-deficient patients should be examined more closely.

APPENDIX TO
ANNUAL REPORT SUMMARY

I. Research Highlights

1. Activated hepatic phenylalanine hydroxylase in the presence of L-tyrosine catalyzes the rapid oxidation of tetrahydrobiopterin (BH_4) with no detectable hydroxylation of the amino acid. The hydroxylase functions as a tetrahydropterin oxidase.

2. Pure hepatic phenylalanine hydroxylase catalyzes the hydroxylation of p-methyl-phenylalanine to p-hydroxymethyl-phenylalanine; molecular oxygen is the sole source of the oxygen in the newly synthesized hydroxymethyl group.

3. We have isolated a cDNA for human dihydropteridine reductase, determined the complete nucleotide sequence, expressed the enzyme in both monkey cells and E. coli and localized the gene to human chromosome 4.

4. Only the subgroup of Alzheimer patients who show movement disorder have decreased CSF levels of BH_4.

5. BH_4 appears to play a role in growth of erythroleukemic cells.

6. Pyruvoyltetrahydropterin reductase has been purified from rat brain. Specific antibodies to the enzyme have been used to show that this enzyme is involved in BH_4 synthesis in brain.

II. Use of Existing Resources

1. Further characterize the brain phosphatase that catalyzes the deactivation-dephosphorylation of phosphorylated tyrosine hydroxylase.

2. Continue to study the possible role of BH_4 in normal hematopoiesis.

3. Study the basis of the high state of activation of phenylalanine hydroxylase in hepatoma cells

4. For each cDNA of phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase, dihydropteridine reductase, and 6-pyruvoyl-tetrahydropterin synthase we will perform the following experiments: 1. Subclone the cDNA into high level expression vectors to synthesize large amounts of material for biochemical studies. 2. Construct deletion mutants to identify regions in the protein that are important from a catalytic or regulatory standpoint. 3. Perform site-directed mutagenesis to precisely map functional sites of the protein molecule and to elucidate the importance of specific residues in the biological role of the enzyme. 4. Exchange domains between certain proteins to gain further insight into the structure-function relationships of these enzymes. 5. Use these

probes to discover the types of mutations in mental disturbances where defective alleles of these genes can play a role, such as manic-depressive disorder. 6. Demonstrate the ability of these probes to have predictive value in screens for such abnormalities as affective disorders.

III. Proposal for New IRP Direction(s) Requiring New Resources

Our endeavors to study the molecular biology and enzymology of the aromatic amino acid hydroxylase systems would be facilitated by an increase in space, manpower, and equipment. A tightly-merged approach affords the best picture of the actual biology in this complex area. An increase in laboratory space, appropriate personnel, and automated equipment would greatly enhance our capabilities. The available equipment that would be beneficial includes devices to purify samples, perform electrophoresis and gel transfers, and DNA scan radioactive gels and filters.

Annual Report
Laboratory of Neurophysiology
October 1, 1987 to September 30, 1988
Steven P. Wise, Acting Chief

I. Overview

LNP research focuses on the functional organization of the primate frontal lobe, particularly the premotor areas. Ours is a concerted, multidisciplinary approach toward studying higher brain functions, behavioral neurophysiology, an approach which demands that strict behavioral control and the highest level of neuroanatomical expertise be combined with physiological techniques.

In recent studies, LNP researchers have compared the activity of cortical neurons while a monkey performs one of several visuomotor tasks, each designed to shed light on the neural mechanisms underlying intentional and anticipatory processes of the brain. We have followed up our finding that premotor cortex neurons show set-related activity, discharging when a monkey has already been instructed about a movement to be made at some future time. We have gained support for our hypothesis that set-related activity reflects the preparation for an upcoming limb movement and that such activity may be at the basis of the behavioral flexibility that characterizes advanced mammals such as primates.

In our most recent work, we have focused on another pattern of premotor cortex activity, which we call anticipatory. This neuronal discharge pattern precedes a temporally predictable stimulus. We found that anticipatory activity is unlikely to reflect any motor control function, per se. Instead, the population of cells showing this activity pattern appears to be part of a mechanism for estimating the timing and/or general nature of predictable events, a finding that calls into question the simple distinction traditionally drawn between motor and supramotor (prefrontal) areas of the frontal lobe.

II. Relevance of the Research Program of the LNP to that of the Institute

Any informed attempt to address the problem posed above leads directly to general questions about, and indeed is informed by, mental health and illness. Failures to adequately adapt to one's environment or prepare appropriately for future actions may lead to a number of mental disorders, especially those involving, directly or indirectly, frontal lobe function. If, as has been hypothesized, certain kinds of mental illness result from failures of the frontal lobe, then one of the most important things we can do for the people afflicted with such disorders is to learn as much as we can about that cortical region, what it does, how it does it, and how it came into being phylogenetically. Following up the hypothesis that schizophrenia may be caused by degenerative changes in the frontal cortex, Melvyn Heyes, a Visiting Associate from the U.K., has begun an examination of the neurochemical consequences of depleting the frontal cortex of dopamine, especially in the basal ganglia. This project includes a frank attempt to develop a primate model of schizophrenia. It is scarcely possible to overestimate the significance to mental health research if current hypotheses about the biological basis of schizophrenia enable us to create the first useful animal model of the disease. It hardly needs to be emphasized, however, that such research is risky and, in the end, has only a small probability of complete

success. But we need only to examine the human costs of the disease (100 times research expenditures, not to mention the immeasurable emotional suffering of both patients and their families) to determine whether it is worth the risk, the time, the effort, the money, and ultimately, the lives of our research animals. Of course, it is! Even if Heyes and his colleagues are not be successful in their attempt to model schizophrenia, their research can, nevertheless, be expected to provide important new information about the chemical interactions between the frontal cortex and the basal ganglia, which will be of acute relevance to other brain diseases.

The other major projects in the LNP are also devoted to gaining a better understanding of the frontal lobe. Shraga Hocherman, a Visiting Associate from Israel, is currently in the early stages of the LNP's first examination of the frontal cortical physiology of complex movements and Steven Wise, a Research Biologist, is conducting the first specific comparison of prefrontal and premotor activity. Study of the prefrontal cortex is a natural extension of our nearly decade-long investigation of the functional organization of the caudal parts of the primate frontal lobe. Other studies of frontal lobe organization and specialization were pursued this year by Andrew Mitz, a Senior Staff Fellow. His work was devoted to the generation of motor maps within the frontal cortex, with particular emphasis, in the past year, on those parts involved in the cerebral control of eye movements. Finally, a new approach to the same general problem is being initiated by our newest Staff Fellow, Chisato Asanuma Stanfield. She is developing a research program aimed at an analysis of the fine, cell-level anatomical organization of the circuits underlying frontal lobe function, specifically circuits within the thalamus that may control selective attention or arousal by regulating the inputs coming to the cortex.

In addition to our main research program, we have collaborated with the Laboratory of Child Psychiatry (Judith Rapoport, Chief) in the development of a speculative hypothesis concerning a possible neurobiological basis for obsessive-compulsive disorder (OCD). A paper based on that hypothesis has recently been accepted as a featured publication in the American Journal of Psychiatry.

III. Research Progress Overview

A. Behavioral Neurophysiology

1. Functional Organization of the Caudal Frontal Lobe. The principal physiological project in the Laboratory of Neurophysiology addresses the functional organization of the frontal cortex. Three full-length refereed paper have already been accepted for publication on the basis of the studies conducted recently, and two more papers will be submitted for publication before the end of this fiscal year.

The first study in this group involves a comparison of premotor cortex activity when the target of a hand movement is indicated directly by illuminating the target and when the same instruction is delivered via abstract or arbitrary visual cues. It was important to pose this question at the level of single cells in awake, behaving animals because only then can neuronal activity be assessed in the absence of distortions introduced by anesthetic agents. Furthermore, only completely alert animals can make meaningful choices among behaviors. We were able to show that cells in one part of the frontal cortex, the premotor cortex,

have as much or greater activity when the cues that guide behavior are abstract or arbitrary as when they are targets to be directly touched or grasped. This finding puts on a physiological basis the hypothesis that the frontal cortex, generally, and the premotor cortex, in particular, are part of a mechanism underlying behavioral flexibility.

The second study involved a comparison of premotor cortex activity with that in the supplementary motor cortex. It has been hypothesized that the frontal cortex divides its labor, with the supplementary motor cortex controlling self-generated behavior and the premotor cortex controlling sensorially-generated behavior. Thus, it has been suggested that animals direct their movements to objects with the premotor cortex and move on the basis of internal representations or memories with the supplementary motor cortex. Our study tested one aspect of this hypothesis: we compared neuronal activity when monkeys made forelimb movements on the the basis of trial-specific sensory instructions vs. activity when the monkey selected a movement without such guidance. We found that most cells in the premotor and the supplementary motor cortex show approximately the same activity when the monkey makes a movement with either sort of guidance. This finding supports our hypothesis that the premotor cortex contributes to the preparation for limb movements regardless of what leads to those movements. However, this finding does not lend much support to the "division of labor" hypothesis outlined above: the premotor and supplementary motor cortex appear to be very similar in their activity and, therefore, are most likely to work together in the selection and control of at least this aspect of behavior. Despite the fact that most cells showed similar activity during a sensorially-instructed and a noninstructed behavioral task, those cells that did show a difference were usually more active when sensory signals instructed the monkey about what behavior to perform. All of the cells with large differences in the two tasks were much more active in the sensorially-instructed task. This finding supports two ideas that the Laboratory of Neurophysiology has championed: first, that each motor cortical field subserves a multiplicity of functions, often simultaneously, and second, that among the contributions of the premotor cortex is an improvement in the animal's capacity to prepare for future actions, especially on the basis of environmental context.

The third neurophysiological study in this group concerned the internal organization of the premotor cortex. Anatomical and published physiological data have been contradictory about whether the premotor cortex has a topographic organization. Accordingly, we sought to reinvestigate this issue with single-unit recording techniques and found that the premotor cortex is indeed topographically organized. The cells involved in the preparation for and execution of foot movements are generally medial to those involved in the preparation for and execution of hand movements. Most importantly, the data show that most premotor cortical cells contribute to specific behaviors, not, for example, the execution of a certain pattern of movement that could be made by either the hand, head or foot.

The fourth neurophysiological study was designed to examine whether a certain neuronal activity pattern, which we term anticipatory discharge, reflects some aspect of motor control. We compared neuronal activity before visual stimuli that indicated both when and where to move the limb and before stimuli that indicated only when to make a limb movement. It was found that most premotor

cortex cells have similar activity in the two situations, thus suggesting that they are principally involved in the timing of behavioral events. This finding further points to the multiplicity of premotor cortex functions, as noted above.

Taken together, the behavioral neurophysiological data are consistent with the hypothesis that the premotor cortex confers the ability to prepare for future events, to select, in advance, an appropriate behavior to address those events, and to act with other cortical fields as an executive in performing the chosen acts.

2. Frontal Cortex Mapping. LNP researchers have used a technique that makes it possible to evoke movements with electrical stimulation from parts of the frontal cortex heretofore resistant to this approach. Mitz and Wise were able to show that the supplementary motor cortex is topographically organized, despite recent published assertions to the contrary. Mitz and Godschalk have now extended that study by showing that the frontal cortical region from which eye movements can be evoked is larger than previously believed. In and near the classical frontal eye field, electrically evoked movements are always of a certain amplitude and direction, whereas more medially, close to the supplementary motor cortex, evoked eye movements converge instead on a given eye position within the orbit. This finding suggests that the medial frontal cortex is important in selecting goals, whereas the lateral cortex is more important for translating those goals into action.

B. Neuroanatomy

Asanuma Stanfield has established the facilities for a fine-grained study of the circuits underlying the interactions between cortex and thalamus, focusing on the thalamic reticular nucleus. Her approach is to study the neuronal morphology of the reticular nucleus as well as its afferents. Although long held to be the final link in an ascending, 'nonspecific', activating system (mediating arousal), recent studies indicate that the reticular nucleus is instead part of a circuit that selectively modulates the transmission of signals to the cortex, including the frontal lobes, and therefore may underlie selective attention. Asanuma Stanfield has discovered that a major input to the reticular nucleus is a presumptively cholinergic projection from the basal nucleus of Meynert. Since these cells degenerate in Alzheimer's disease, it is possible that some of its symptoms arise from failure of information gating normally mediated by the thalamic reticular nucleus.

C. Neurochemistry

Heyes has approached two general problems: what neurochemical changes in the basal ganglia are caused by depletions of frontal cortex dopamine in rats and monkeys, and, more generally, what is the biochemical basis for neural degeneration. An examination of neurochemical changes in basal ganglia after specific neurochemical depletions in the frontal cortex is part of an explicit attempt to develop an animal model for schizophrenia. To date, studies in rats have shown that depletion of dopamine in the frontal cortex leads to an increase in dopamine in the nucleus accumbens, a very encouraging finding. Preliminary, and we stress, highly preliminary, results of similar studies in primates yield a similar conclusion regarding other parts of the striatum. It will now be necessary to increase the number animals in each group to allow valid statistical comparisons to be made. As for the general problem of neural degeneration, Heyes has

developed a specific and highly sensitive assay for the naturally occurring neurotoxin, quinolinic acid. He has found, for example, that quinolinate levels are elevated in the striatum of patients with certain types of Parkinson's disease.

IV. Proposed Course of the Laboratory

In the past three years, the LNP has seen the most radical changes in its over-thirty-year history. Our change in focus has enabled us to address higher brain functions in addition to the relatively simple behaviors that have traditionally been studied by motor systems physiologists. We now study the brain mechanisms underlying complex motor trajectories, flexible switching between behaviors on the basis of different guiding rules, and physiological changes accompanying motor learning.

Dividing the laboratory and moving it to the NIH Animal Center, which we did in 1987, enabled us to update the LNP's physical facilities to the new levels required by ever-increasing regulation of nonhuman primate research, and did so much more quickly than the NIH's AALAC-accreditation plan. However, we were concerned about whether the LNP could attract the breadth and level of scientific talent that has characterized the laboratory of Wade Marshall, Paul MacLean, Ed Evarts, Mahlon DeLong, Emilio Bizzi, Tom Thach, Peter Strick, Stanley Rapoport, Fred Miles, Steve Lisberger, Yoshi Shinoda, and Jun Tanji, to name but a few. However, recently, the LNP has once again begun to attract scientists of the first rank. Our approach, has been to gather together researchers to support the triad that makes up behavioral neurophysiology today: anatomy, physiology, and behavioral biology, each with an independent research program, but each contributing to the dept and breadth of the LNPs main research program on the functional organization of the primate frontal lobe. Chisato Asanuma Stanfield, a skilled and well-rounded neuroanatomist, gives us the breadth of knowledge in that most important, but oftimes neglected, aspect of behavioral neurophysiology. At the same time, she has begun an independent project that seeks to study the detailed microcircuitry underlying selective gating of information flow through the thalamus (to cortex), a natural follow-up to her classic work on the circuits relaying subcortical information to the frontal cortex. Timothy Barth, is an outstanding experimental psychologist who will join the laboratory in July, will be able to contribute his understanding of animal behavior to the laboratory's expertise. At the same time, he will be able to continue his studies on cortical plasticity in the somatosensory and motor cortex of rodents and the effects of drug treatments on recovery of function after brain lesions. Finally, we expect the arrival in the summer of Giuseppe di Pellegrino, an Italian neurologist, who was so motivated to work in the laboratory that he will support himself until one of the two grants for which he has applied is funded. Our current investigators are themselves not without certain laurels. Mitz, a true physiologist and an important member of the laboratory for reasons not always apparent on a curriculum vita, has been awarded a highly competitive NATO Grant for International Collaboration with Moshe Godschalk of Erasmus University in The Netherlands, a former Visiting Associate in the LNP.

As for specific research plans of the laboratory, our current studies focus on (a) the differences between neuronal activity in the prefrontal and premotor cortex, (b) set-related activity before complex, curved movements and simpler, straight movements, (c) changes in set-related activity as the monkey learns new

visuomotor associations, (d) inputs to the reticular complex of the thalamus, and (e) frontal corticostriatal interactions and their effect on dopamine metabolism. These projects tests specific hypotheses about functional specializations within inputs to, or outputs of the frontal cortex, drawn from our own, clinical, or neuropsychological research, which suggest that premotor and prefrontal areas, respectively, function at increasingly higher hierarchical levels. Explorations of these hypothesis will do much to improve our understanding brain mechanisms, especially those involved in the selection and control of behavior.

V. Appendix to the Annual Report Summary

1. Research Highlights:

A. The first physiological indication that the traditionally-drawn dichotomy between prefrontal and premotor cortex function in terms of supramotor vs. motor functions is not valid. Parts of the motor cortex perform nonmotor roles in addition to their more well-established motor functions.

B. The premotor and supplementary motor cortex are not specialized for behaviors either emitted in response to sensory signals or memories, as hypothesized by others, but instead work together in the selection and control of both types of behavior.

C. Depletions of frontal cortex dopamine may, as indicated in highly preliminary analysis of pilot studies, cause the changes in striatal dopamine levels predicted by one influential theory of schizophrenia.

2. Plans for Immediate Future (Using Existing Resources):

A. An elaboration of the pilot attempt to develop a primate model for schizophrenia by neurochemical manipulations of the frontal cortex.

B. The first direct comparison of physiological activity in the prefrontal vs. premotor cortex, and a continuation of the microstimulation mapping study of the frontal cortex.

C. The first physiological study of motor memory retrieval in monkeys.

3. Proposal for New Directions (Requiring New Resources):

A. We hope to develop methods for monitoring neuronal activity of free-ranging monkeys. Specifically, we hope to study frontal cortex activity during species-typical behaviors when they are triggered by several different events: spontaneously by visual stimuli, under operant control, and by stimulation from conspecifics. New resource requirements would include technical development, increased support of animals maintained in social groups, and support of a Fogarty Visiting Fellow.

B. We would need support to develop a multielectrode single-unit recording system, which is currently the state-of-the-art in behavioral neurophysiology.

Report of the Chief of the Neuropsychiatry Branch
Intramural Research Program
National Institute of Mental Health

The Neurospsychiatry Branch has been aggressively pursuing research that will answer the troubling questions about the cause, treatment, and prevention of schizophrenia. Also, our staff continues to provide exciting knowledge in neurovirology, neuroimmunology, molecular biology, neurografting, and neurostimulants.

In animal models of Parkinson's disease and epilepsy, we have improved grafting procedures in the rat and primate. In the nonhuman primate, we have been able to achieve successful adrenal autografts with our recently developed stereotaxic instrument. This makes possible gentle and precise insertion of grafts into the brain with survival being significantly greater than we have previously achieved.

During the past reporting year, Dr. William Freed has made significant progress towards development of a feline model of Parkinson's disease to explore neurografting in an animal larger than the rat, but less expensive to use than nonhuman primates. Dr. Freed has also been investigating the factors that may influence the efficacy of brain grafts in animal models of Parkinson's disease. For example, he has found that embryonic substantia nigra grafts appear to be more effective in immature hosts than the mature animal. Brain injury also appears to increase the degree of innervation of host brain by grafts. Adrenal medulla grafts, on the other hand, appear to be influenced by adrenalectomy of the host animal. These findings may assist in the development of more effective transplantation procedures.

A second area of investigation that Drs. Freed and Poltorak, in collaboration with Dr. Geller of Rutgers University, are pursuing is the transplantation of defined cell types into the brain. Three catecholamine-producing cell lines have been investigated--Pc12 cells, B16/c3 melanoma cells, and N115E neuroblastoma cells. These cells have all been found to undergo significant morphological changes following intracerebral transplantation. The N115E cells appear to express tyrosine hydroxylase and show the most stable, persistent characteristics. We hope that these cells will serve as a model for future use of genetically engineered cell lines. One cell type, a rat central nervous system (CNS) glial cell line immortalized by a retroviral vector, has been developed by Dr. Geller. These cells have already been characterized in terms of in vitro growth properties and expression of glial markers. Studies of these cells following intracerebral transplantation have recently been initiated.

Dr. Freed has continued to investigate the behavioral role of the "quisqualate" type of excitatory amino acid receptor. He has conducted studies of the role of these receptors in seizures, as well as changes in the behavioral effects of drugs that interact with these receptors in animals chronically treated with neuroleptic drugs. Dr. Freed has developed a hypothesis suggesting that neuroleptic drugs produce their antipsychotic effect primarily by producing changes in the quisqualate receptor at corticostriatal terminals. Studies to directly test this hypothesis are now under way.

Dr. Maciej Poltorak has also been conducting studies of normal and abnormal brain and intracerebral neuronal transplants using markers for neurofilaments, cell adhesion markers, and immunological marker antigens. He has used these markers

to compare brain grafts in differing host brain environments and to investigate brain tissue from patients with schizophrenia and in mice exposed to alcohol during fetal development. Dr. Poltorak has also made advances in characterizing the process of intracerebral grafting rejection using specific immunological markers.

Dr. Janice Stevens has continued her exploration of the feasibility of brain grafts of GABAergic tissue to specific brain areas in rat models of epilepsy. These are among the first trials using brain transplants in the search for a new treatment for this disorder, which affects about 0.5 percent of the United States' population. She has also continued her search for a viral etiology of schizophrenia through examination of the cerebrospinal fluid (CSF) and postmortem brain tissue of schizophrenic patients. Because of encouraging results, new studies inoculating newborn rodents with CSF from schizophrenic patients and controls began in March and will be continued this year. Immunocytochemical studies of frozen and fixed brain specimens are simultaneously being pursued in collaboration with Dr. Maciej Poltorak.

Meanwhile, Dr. Anita Feenstra finished two studies in which lymphocyte cultures of schizophrenic patients and normal controls were tested for the presence of retrovirus. In the first study, cultures were grown under conditions that induce the expression of known human retroviruses. In the second study, the cultures received an additional treatment with 5-azacytidine, which reactivates some transcriptionally silent genes. Both studies, however, failed to provide evidence for the presence of a retrovirus in the peripheral lymphocytes of schizophrenia. In addition, serum of schizophrenic patients was tested for the presence of antibodies to the newly described human virus, Human B-cell Lymphotropic Virus (HBLV). Our patient sample was not different from a normal population, and we tentatively concluded that HBLV does not play a role in the etiology of the disorder.

We established a P-2/3 laboratory, headed by Dr. Rita Anand, to investigate the biological properties of the human immunodeficiency virus HIV-1. The focus of her studies is to understand the biological effects of retroviral infections in the central nervous system. Specific aims of this lab are to: find out the generality of prevalence of noncytocidal HIV-1 isolates in neuropsychiatric HIV-1+ patients; determine if such noncytocidal variants mutate, change to cytocidal viruses, and cause immunodeficiency syndrome; and localize the genetic determinants of neurotropism and noncytopathicity. Dr. Anand has studied the cellular tropism of these isolates since the laboratory was established in December, and has found that noncytocidal variants replicate to significantly higher levels in monocytic cell lines than T4+ lymphocytes. She has also observed low levels of replication of these isolates in cell lines of neuronal and glial origin. Molecular characterization, including DNA sequencing and computer analyses for homologies, has been completed for the env, nef, and LTR of one of the isolates ($HIV\text{-}1_{BR}$) from a patient who died of progressive dementia. The knowledge acquired in these studies will further our understanding of the mechanisms of retroviral-induced neuropsychiatric disorders.

Dr. Anne Marie Duchemin, together with Drs. Tam Quach and Bruce Schrier, has continued her project on the molecular cloning of a lesion-induced neurotrophic factor. Screening of a cDNA library from a mRNA fraction, selected by its ability to induce neurotrophic factor synthesis in xenopus oocytes, has lead to selection of several positive clones. DNA sequencing and analysis of the fusion protein of these clones is in progress. Dr. Duchemin has also observed that the CSF of some schizophrenic patients has higher neurotrophic activity for

sympathetic neurons than that of non-schizophrenic patients and normals and that this higher activity is independent of neuroleptic treatment. It is, however, positively correlated with ventricular enlargement of the brain as measured on the computed tomography scan. Unfortunately the bioassay we have used produces variable results from day to day. To overcome the poor reliability of the original assay, neurite-promoting activity of CSF on the neuroblastoma cell line (with high reliablity) was tested by Paul Oliver. Regrettably, no difference between the CSF of schizophrenic patients and controls was found. Differences in these two assays are now being explored.

Dr. Duchemin has also continued her studies of gene expression regulation in rodent brain. In collaboration with Dr. Michael Iadarola, she determined the regional distribution of cholecystokinin mRNA as well as the peptide in discrete areas of mouse and rat brain and made a comparison with enkephalin mRNA levels in the same regions. Two unexpected results were obtained from this study: the thalamus was found to be a region of CCK synthesis, while striatum, which contains high levels of the CCK peptide, had a very low level of CCK mRNA.

Last year Dr. James Lohr demonstrated that Vitamin E produced about a 50 percent decrease in tardive dyskinesia movements. Dr. Michael Egan has continued this clinical study of the efficacy of antioxidant therapy for tardive dyskinesia. He is performing the first inpatient trial of vitamin E, giving us better control of both medication compliance and patient evaluation. He has screened four of 40 patients who have entered the 12-week, double-blind, placebo-controlled study, which involves looking at the effects of vitamin E on Brief Psychiatric Rating Scale scores, negative symptoms, and self perception of and objective ratings of abnormal movements. While it is too early to give preliminary results, Dr. Egan is encouraged. With the guidance of Drs. Freed and Poltorak, he is examining the neuropathology of IDPN-induced movement disorder in mice and some inter-strain differences in susceptibility to movement disorders.

Dr. Darrell Kirch has extended his work both on the basic and clinical levels of studies related to the neuropharmacology of a number of psychoactive drugs. While expanding his clinical data regarding the pharmacokinetics of haloperidol (including its interactions with other drugs such as ascorbate and the retinoids), he has initiated a series of intriguing studies that are revealing significant interactions between both nicotine and caffeine (two substances commonly abused by schizophrenic patients) and CNS dopaminergic function. He has expanded upon his earlier study of polydipsia and hyponatremia in schizophrenic patients; is now conducting a magnetic resonance imaging study to search for structural abnormalities in these patients; and is completing a trial of demeclocycline as a treatment for this syndrome. He also is an active participant in our expanding studies of neurovirology and neuroimmunology, and in particular has focused on studies of CSF immunoglobulins and plasma interferon as potential markers of infection and/or autoimmunity in schizophrenic patients. While actively pursuing these scientific interests, Dr. Kirch became Medical Director of our newly reorganized clinical program, the NIMH Neuropsychiatric Research Hospital. He is the primary individual responsible for establishing and maintaining a high standard of patient care by our Medical Staff Fellows, the nursing staff, and other professional personnel actively coordinating this clinical care with our research goals.

Dr. Farouk Karoum has continued his experiments on the metabolism of D- and L-dopa in rats, finding that these two substances are equally efficient in producing dopamine. He is now exploring the underlying mechanisms responsible for the formation of dopamine from D-dopa and its effects on peripheral biogenic

amines in the hope of eventually using D-dopa in the treatment of Parkinsonism. Dr. Karoum's further work relating to total body turnover of catecholamines in depression, schizophrenia, and hyperactivity in children using biochemical analyses performed by combined gas-chromatographic mass-spectrometric methods developed in his laboratory has also yielded interesting results. Data from this investigation raise the possibility that schizophrenia may be associated with an imbalance between noradrenergic and dopaminergic systems and that neuroleptic medication preferentially stimulates dopamine turnover, thereby balancing the activity between the two amines.

Dr. Richard Suddath, in collaboration with Dr. Karoum, is involved in studying the biochemical and behavioral effects of chronic cocaine administration in animals. The finding of persistent depletion by cocaine on dopamine metabolites in the frontal cortex has lead to studies of the effects of chronic cocaine on receptor physiology, neuropeptide levels, and immunohistochemistry. In process is a study of regional cerebral blood flow and peripheral dopamine metabolites in cocaine addicts undergoing withdrawal. Dr. Suddath's research also includes structural neuroimaging in schizophrenia by means of magnetic resonance imaging and a computerized image analysis system. He recently reported a reduction in temporal lobe gray matter of patients with schizophrenia, a reduction that correlated with ventricular enlargement.

In his third year with us, Dr. Gregory Straw has maintained a high level of diverse activities. He has furthered his investigation of the pathophysiology of retinoic acid action in vivo. He has found that 13-cis-retinoic acid decreased the serum haloperidol concentration in schizophrenic patients. Furthermore, his project involving calcium channel inhibitors has shown nifedipine to cause a trend toward improvement in abnormal movements in schizophrenic patients. An analysis of seasonal variations in birth and admission rates for schizophrenic patients has shown a significant but small (5 percent) yearly cyclicity in admissions of schizophrenics to Saint Elizabeths.

Open clinical trials of isotretinoin in schizophrenic patients produced an initial dose response curve needed for placebo-controlled trials. A placebo-controlled, double-blind, crossover study of isotretinoin in schizophrenic patients on and off haloperidol will yield useful information regarding the efficacy of this drug to improve present treatments of psychotic symptoms. Dr. Straw has found that retinoic acid does alter the tissue distribution of haloperidol in the rat, and that it alters some neurotransmitter levels and turnover in the rat brain. He has produced data that demonstrate diverse and developmentally dependent behavioral changes in rodents after acute and subchronic retinoic acid treatment. In addition, he has developed mathematical models for the time course of rodent exploratory locomotor habituation.

Dr. Myles Jaffe is continuing his investigation of the pharmacology and biochemistry of the retina, which has shown that a D-2 blocker attenuates both rod and cone activity of humans. Diazepam also has an attenuating effect, more pronounced under conditions of light-adaptation.

Dr. Robert Alexander's work reviewed the relevant data from phenomenologic, laboratory, treatment, outcome, and family and genetic studies to settle the debate that continues over whether bipolar disorder and schizophrenia represent distinct categories of illness or exist on a continuum of psychosis, separated by degree of symptom expression, not underlying etiology. He concluded that

schizophrenia and bipolar disorder are best conceptualized as two distinct disorders.

Sharing some of the highlights of our outreach efforts, we have had 10 guest researchers working with our regular staff, have published or have in press 80 papers, enjoyed a number of specially scheduled outside speakers, and participated in about 75 conferences and speaking engagements nationally and internationally in addition to our ongoing creative scientific research endeavors. Also, together with Drs. Martin Peckerar of the Naval Research Laboratory and Shabit Shamma of the University of Maryland, we sponsored the Second Workshop on Synthetic Microstructures in Biological Research at Airlie House, Virginia. The meeting had an international turnout of academic and commercial scientists, who enthusiastically endorsed a third workshop for 1989. Please let me note the accomplishments of Dr. William Freed, who received the 1987 PHS Superior Achievement Award.

Amidst so much productivity, we are sorry to be losing some of our staff, though we know they will continue to achieve important things in their new environments. Best wishes to Drs. Anne Marie Duchemin, Myles Jaffe, and Evan DeRenzo. We also bade farewell to Willie Turner.

On the other hand, we welcome Drs. Ana Hitri and Helena Kulaga, and trust that their time at NIMH will be both productive and enjoyable. We also welcome Ms. Christina Wynn to our secretarial staff. Dr. Sanjeev Nayar happily joins our P-2/3 laboratory, and Nancy Long and Norman Kane join our technical staff. We look forward to our two new staff associates, Drs. David Glovensky and Vikram Khot, as they develop into excellent scientist clinicians. We are extremely pleased to have such a fine staff.

Annual Report
Laboratory of Preclinical Pharmacology
October 1, 1987 Through September 30, 1988
H.-Y.T. Yang, Ph.D. Acting Chief (Oct. 1987 to Apr. 1988)

This report covers only the works of the Group on Receptor Pharmacology headed by D.-M. Chuang, Ph.D. and the Section on Neuropeptides headed by H.-Y.T. Yang.

The Group on Receptor Pharmacology (Chief, Dr. Chuang) continues to focus their study on characterization and regulation of neurotransmitter receptors which are coupled to phospholipase C and adenylate cyclase by using primary culture of brain cells and neurohybrid tumor cells. Using primary culture of cerebellar granule cells, they have demonstrated (1) the presence of phospholipase C coupled muscarinic cholinergic, α_1-adrenergic, H_1-histaminergic, glutamatergic and 5-HT_2 receptors and the release of neurotransmitter glutamate by activation of these receptors, (2) the development of homologous receptor desensitization after prolonged receptor stimulation and (3) potentiation of the efficacies of excitatory amino acid and cholinergic receprtor agonists after long-term exposure of the cells to GABA. Using NCB-20 cells, the group has detected a novel adenylate cyclase linked 5-HT receptor. They are currently extending this study to determine whether the same receptor exists in the CNS and, if so, the role of this receptor in neuronal function. The group has also found that phospholipase C in NCB-20 cells can be activated by stimulation of muscarinic cholinergic, H_1-histaminergic and bradykinin receptors or activators of voltage-sensitive sodium channels such as veratridine and bachachotoxin suggesting a role of phospholipase C in synaptic transmission. Using a related neurohybird cell lline, NG108-15, development of morphine tolerance was investigated by studying cAMP system. The group also continues their study on β-adrenergic receptor desensitization using C_6-glioma cells and hopes to uderstand whether internalization and desensitization of β-receptor are associated with transcription rate of β-receptor mRNA. Their preliminary results using β_2-receptor cDNA have revealed that stimulation with isoproterenol causes a rapid decrease of β_2-receptor mRNA.

The section on Neuropeptides continues to focus their study on the putative antiopiate peptide, phe-leu-phe-gln-pro-gln-arg-phe-NH_2 (F-8-F-NH_2). In this study, the emphasis was placed on regions where there are high levels of F-8-F-NH_2 with the aim of further delineating the functional role of F-8-F-NH_2. High concentrations of F-8-F-NH_2 are present in dorsal spinal cords, periaqueductal gray areas and pituitary glands. Release of F-8-F-NH_2 from spinal cords was studied. The results to date indicate that F-8-F-NH_2 can be released from the spinal cord and substance P may have a role in this release. Studies on the pituitary have shown that F-8-F-NH_2 levels in pituitary glands of Brattleboro rats are below the limit of detection suggesting that there may be an interaction between F-8-F-NH_2 and vasopressin in the pituitary gland. Immunohistochemical studies have revealed that, in periaqueductal gray areas, F-8-F-NH_2 is present in fibers and nerve terminals but not in cell bodies. Immunohistochemical studies have revealed two strong groups of F-8-F-NH_2 positive cell bodies in the

periventricular hypothalamic area and nucleus tractus solitarii. Whether F-8-F-NH_2 positive nerve terminals in substantia gelatinosa of spinal cords and pituitary glands originate from these cell bodies is under current study. The section has also continued their study on adrenal NPY. We have previously detected a new NPY-like peptide in adrenal glands of bovine and old rats. This peptide is not present in adrenal glands of young rats. This NPY-like peptide has now been purified to homogeneity and biochemically characterized. The final confirmation of the structure determination is in progress.

The following summaries of the years research activities have been provided by group leaders.

Group on the Receptor Pharmacology (Head: D.-M. Chuang, Ph.D.)

The research program in the group on Receptor Pharmacology lies in two major areas: (1) characterization of neurotransmitter receptors coupled to phospholipase C and adenylate cyclase in neurons and related neuronal tissues; (2) molecular mechanisms of the regulation of second messenger production mediated by these receptor-coupled effectors.

Using primary culture of cerebellar granule cells, Dr. Chuang's group has found that these cells express muscarinic cholinergic, α_1-adrenergic, H_1-histaminergic, glutamatergic and 5-HT_2 receptors coupled to phosphoinositide hydrolysis by phospholipase C. Two key products of this enzymatic reaction are diacylglycerol and inositol trisphosphate which by activating protein kinase C and mobilizing intracellular calcium, respectively, trigger an array of physiological responses. Cerebellar granule cells in culture mature into excitatory neurons which synthesize and release the transmitter glutamate. They have found that the release of glutamate from granule cells is enhanced by stimulation of these phospholipase C-coupled receptors. Moreover, this effect on glutamate release can be mimicked by direct activation of protein kinase C with phorbol ester, suggesting that the diacylglycerol/protein kinase C arm of phosphoinositide metabolism may be involved in the synaptic transmission of cerebellar granule cells in vivo. At present, Ms. Dillon-Carter (a chemist) and Dr. Chuang are studying the desensitization of phospholipase C-coupled receptors in these cells. They found that prolonged stimulation of muscarinic cholinergic, α_1-adrenergic, H_1-histaminergic and 5-HT_2 receptors with their selective agonists caused a time-dependent desensitization of their respective receptors to subsequent stimulation with the desensitizing agonist. However, the phosphoinositide response mediated by the receptors which were not prestimulated remained virtually unaffected, thus indicating that the desensitization is homologous. In contrast, pretreatment of cells with phorbol esters induced a rapid and robust heterologous desensitization of all these receptor-mediated activation of phospholipase C. Currently, they are investigating the role of protein kinase C and other forms of kinase in the process of agonist-induced desensitization. Prolonged agonist stimulation also leads to loss of the receptor binding sites coupled to phospholipase C. The mechanisms underlying this agonist-induced receptor loss and its role in the desensitization

process are also under investigation. Dr.'s O.-F. Yu (a NRC associate) and Chuang have studied the inter-regulation between receptors coupled to different effectors in granule cells. They found that long-term exposure of cultured granule cells to micromolecular concentrations of γ-aminobutyric acid (GABA) markedly enhanced the efficacies of excitatory amino acids including L-glutamate, N-methyl-D-aspartate and quisqualate and the muscarinic cholinergic receptor agonist carbachol to stimulate the turnover of phosphoinositide. The basal turnover rate as well as the activity stimulated by kainate and norepinephrine were unchanged, thus indicating the selectivity of this modulation. Since the pharmacological effects benzodiazepines is believed to be mediated by facilitation of GABA transmission and chronic benzodiazepine treatment has been reported to affect glutamatergic and cholinergic neurotransmission, their findings of effects of chronic GABA exposure on granule cells may have implications for the molecular mechanisms of tolerance and dependence elicited by long-term administration of benzodiazepine.

Dr. Chuang has extended his studies on the regulation of phospholipase C activity in a neuroblastoma hybrid cell line, NCB-20. He has found that the phospholipase C activity in this clonal cell line can be activated by stimulation of muscarinic cholinergic, H_1-histaminergic and bradykinin receptors. These receptors are coupled to distinct pools of phosphoinositides and do not show cross-desensitization when prestimulated with a selective receptor agonist. In addition, Dr. Chuang found that activators of voltage-sensitive sodium channels such as veratridine and bachachotoxin markedly stimulate the activity of phospholipase C in NCB-20 cells. This modulation is dependent upon the presence of calcium but independent of sodium in the medium, suggesting that influx of sodium is not a prerequisite for this activation. Moreover, veratridine can selectively inhibit phosphoinositide hydrolysis mediated by muscarinic receptor stimulation. His finding supports the notion that voltage-sensitive sodium channel, muscarinic receptor, phospholipase C and a GTP binding protein of unknown identity is a complex of interacting molecular entities. In view of the fact that depolarization of neurons is often associated with activation of voltage-sensitive sodium channels, the observation that phospholipase C activity in NCB-20 cells can be activated by veratridine and batrachotoxin may have fundamental implication for the role of phospholipase C in synaptic transmission. NCB-20 cell line is known to express a serotonin (5-HT) sensitive adenylate cyclase. However, the nature of the 5-HT receptor involved in this activation is unclear. Using new and selective 5-HT agonists and antagonists, Dr.'s J. Cossery (a visiting fellow), A. Mellow (a guest worker) and Chuang have undertaken the characterization of the cyclase-linked 5-HT receptors in this cell line. They found that only 5-HT, 5-methoxytryptamine and the selective 5-HT_3 agonist 2-methyl-5-hydroxytryptamine produced a dose-dependent increase in cyclic AMP level in NCB-20. Putative 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} agonists were ineffective in producing this increase. Interestingly enough, 5-HT_3 receptor antagonists ICS-205-930 and MDL 72222 were virtually inactive in blocking the 5-HT-induced activation of adenylate cyclase. Their available data suggest the existence of a novel 5-HT_3-like receptor linked to the cyclase. Currently they are studying whether this previously undescribed 5-HT_3-like receptor is present in the CNS and, if so, the neurophysiological role of this new 5-HT receptor subtype. Using a related neurohybrid cell line NG108-15, Dr.'s R. Copeland (a guest worker)

and Chuang have attempted to study whether changes of the basal level of cyclic AMP following morphine treatment can be used as a model for the development of morphine dependence. They have found that stimulation of δ opioid receptors in NG108-15 cells by morphine caused an initial but transient depression of the cyclic AMP. However, approximately 36 hours after morphine exposure, there was a significant rebound increase of the cyclic AMP content. This rebound increase can be inhibited by chloramphenicol, suggesting an involvement of macromolecular synthesis in this rebound effect which may be a suitable model for morphine dependence.

Dr. Chuang's group has previously shown biochemically and immunohistochemically that desensitization of β-adrenergic receptors induced by prolonged agonist stimulation with isoproterenol in a model system of frog erythrocytes is associated with internalization of the β-receptor recognition sites from the plasma membrane and that this receptor internalization is intimately coupled to the desensitization of β-receptor-coupled adenylate cyclase. Dr's C. Hough and Chuang have further addressed the question of whether internalization and desensitization of β-receptors may involve a change in the steady state and transcription rate of β-receptor mRNA. For these studies, they are using the receptor system present in C_6-glioma cells which express both β_1 and β_2-adrenergic receptors with a rapid turnover rate. Their preliminary results using β_2-receptor selective cDNA show that, indeed, β_2-receptor mRNA levels decreased rapidly in response to stimulation with isoproterenol. In addition, they are investigating whether down-regulation and desensitization of β-adrenergic receptors in the CNS following chronic antidepressant treatment is associated with alteration of the receptor mRNA turnover. Their ultimate goal is to elucidate transsynaptic factors involved in turning -on and -off of the expression of genes for β-adrenergic receptors in the CNS and to relate this gene regulation to the action of psychoactive drugs and some forms of mental illnesses.

In conclusion, Dr. Chuang's group has explored several avenues in an attempt to study the regulation of the production of receptor second messengers. Their findings have increased our understanding of molecular details involved in these regulatory processes and may help develop new pharmacological tools that can prevent, alleviate or cure some disease states that are related to receptor malfunction.

Section on Neuropeptides:

The section on neuropeptides continues to extend their study on the neuropeptide, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2). F-8-F-NH_2, which has been shown to attenuate morphine analgesia, is unevenly distributed in central nervous system of bovine and rat brains with the highest concentrations in dorsal spinal cords and periaqueductal gray areas, regions known to be important for the opiate mediated analgesia. Using in vivo (by Dr. Yang in collaboration with Dr. Jhamandas, Queen's University, Canada) and in vitro (by Ms. Majane) superfusions of rat spinal cords (by Ms. Majane), release of F-8-F-NH_2 was studied. It was found that F-8-F-NH_2 can be released by depolarizing concentration of KCl or

substance P, a sensory neurotransmitter. The result seems to further suggest that F-8-F-NH_2 may have a modulatory role in antinociception.

In rats, very high concentration of F-8-F-NH_2 immunoreactivity was detected in pituitary glands and furthermore the immunoreactivity was present exclusively in posterior lobes. Because of this, the physiological role of F-8-F-NH_2 in the pituitary glands was investigated (by Ms. Majane). F-8-F-NH_2 levels in pituitary glands of Brattleboro rats were found to be below the limit of detection. In contrast, normal levels of F-8-F-NH_2 were observed in spinal cords of Brattleboro rats. This result seems to suggesst that there may be an interaction between F-8-F-NH_2 and vasopressin which is known to be absent from Brattleboro rats. The result further suggests that the Brattleboro rat may be an useful model in which to explore the role of pituitary F-8-F-NH_2.

Previous immunohistochemical study has found that F-8-F-NH_2 immunoreactivity is present in nerve terminals of the superficial laminae of the dorsal spinal cord. In this study, locations of F-8-F-NH_2 immunoreactivities in periaqueductal gray areas (by Dr. Salminen) and pituitary glands (with collaboration of Dr., Panula, University of Helsinki, Finland) were studied immunohistochemically. In the periaqueductal gray, using both polyclonal and monoclonal antibodies, F-8-F-NH_2 immunoreactivity was found to be localized in nerve fibers running vertically throughout this area. In horizontal sections, F-8-F-NH_2 immunoreactive nerves were found to form dense networks in posterolateral periaqueductal gray areas. These F-8-F-NH_2 positive nerve fibers seem to originate from other parts of the brain as no immunoreactive cell bodies were detected in the periaqueductal gray. In pituitary glands, F-8-F-NH_2 positive fibers and nerve terminals were detected in posterior lobes. In order to study the sources of these immunoreactive nerve terminals, locations of F-8-F-NH_2 positive cell bodies were investigated (with collaboration of Dr. Panula, University of Helsinki, Finland). Two strong F-8-F-HH_2 positive neuronal groups were revealed in the brain. One of them was located in periventricular hypothalamic area and another prominent cell group was found in nucleus tractus solitarii. Whether these cell bodies project to pituitaries and spinal cords is under current investigation with lesion techniques.

The section also continues their study on adrenal neuropeptide Y (NPY). Previously, it has been found that besides NPY there is an additional NPY-like peptide in adrenal glands of bovine and rat. In rat, this peptide is present in adrenal glands of older rats but not in that of younger rats. The biological implication of this observation is not clear at present time. In this study, NPY-like peptide has been isolated and partially biochemically characterized. A high degree of homology with NPY was observed and the final confirmation of the structure is in progress. In order to study the possible role of NPY-like peptide in the adrenal catecholamine function, primary culture of rat adrenal chromaffin cells has now been established (by Dr. Shimoda). Characterization of this primary culture of rat chromaffin cell during the course of culture is in progress.

Annual Report of The Clinical Brain Disorders Branch
National National Institute of Mental Health
October 1, 1987 - September 30, 1988

Daniel R. Weinberger, M.D., Chief
Joel E. Kleinman, M.D., Ph.D., Deputy Chief

Introduction

The Clinical Brain Disorders Branch, CBDB has completed its first full year of existenc. It has been a very productive and gratifying year. Most of the construction on our wards and laboratories has been completed. We have been very successful in recruiting first rate scientists to spearhead high priority projects. We have at long last finalized our contract with the District of Columbia to provide neurological consultation to patients at Saint Elizabeths hospital. This contract made it possible for us to recruit Dr. Thomas Hyde, chief resident at Standford University, as the neurologist for this program. In addition to our having access through this program to a vast patient population we have been authorized by the District of Columbia to provide an academic program that will make it possible for us to have scientists from around the country visit and discuss their work with us. Other highlights of our recruiting efforts include Dr. Alan Braun to head our nuclear medicine projects and Dr. Richard Saunders who will direct primate research.

Clinical Studies Section

The big news from the clinical studies section is that the SPECT scanner has been operational for approximately six months. There were a number of expected glitches that needed to be smoothed out. In some cases, considerable software development was required. For example, in order to do dynamic rCBF studies, a time correction procedure was created which had not previously been available. Dr. Jones developed an algorithm that made the necessary correction and the result was the finest Xenon 133 tomography images yet produced with this technology. One of the major reasons to pursue SPECT was the possibility of doing rCBF studies with Xenon 127, an isotope of Xenon with far greater potential. At the Society of Nuclear Medicine annual meeting in San Francisco, we presented the first data about this method, illustrating that it can be done and that it is far superior to the standard method with Xenon 133. The presentation generated worldwide interest. This work will establish noninvasive rCBF with Xenon 127 as the nonPET procedure of choice for studying rCBF during life and particularly during cognitive activation.

In addition to studies of rCBF methodology, we have completed Xenon 133 studies with SPECT looking at the effects of the catecholeamine agonist amphetamine on cerebral blood flow. We have continued to look at prefrontal CBF using our old two dimensional probe system and have broadened our understanding of our findings of prefrontal hypometabolism in schizophrenia. In particular, we continue to find that other disorders with cognitive dificits do not look like patients with schizophrenia, suggesting a possibly unique pathophysiological mechanism in the latter. Patients with Down's syndrome are not hypofrontal during the cognitive conditions that result in hypofrontality in schizophrenia. Patients with psychotic affective syndromes also do not show prefrontal reduced rCBF.

We have also begun to explore in vivo neurochemistry with SPECT. We have completed the largest series of studies in the world of normal individual and patients with Alzheimer's disease using I 123 QNB, a selective muscarinic receptor ligand. The images are better than we expected and represent the state of the rt with SPECT. The preliminary data suggest that abnormalities can be seen in every case of Alzheimer's disease. Possibility that this might prove of diagnostic significance is being explored. Studies of brain morphology in schizophrenia remain a major priority. Most of our recent efforts have focused on three dimensional computerized analysis of high resolution MRI scans. Our studies have shown that temporal grey matter is reduced by 15% in patients and that this reduction in cortical grey matter volume predicts cerebral ventricular enlargement. Preliminary results from studies of monozygotic twins discordant for schizophrenia suggest that morphological abnormalities are more widespread than had previously been appreciated.

Our neuropsychology group under the direction of Dr. Terry Goldberg continues to probe cognitive function in schizophrenia and other disorders. The questions asked center around the notion of characterizing which functional neural systems work and which do not. To this extent, this effort works in parallel with our functional imaging projects. Using differential paradigms, the data continue to implicate prefrontal neural networks as the most consistently involved cite.

Section on Neuropathology

The neuropathology section still awaits completion of the primate and autoradiography laboratories. In the meantime, this section has been extremely active and productive. The computerized analysis system for MRI resides in the neuropathology laboratory and this has been used to explore a large number of anatomical areas in the brain in schizophrenia and Alzheimers Disease. While the primarily pathology of the latter is found in temporal lobe, the question of whether the former involves focal or generalized pathology is open. Using a novel technique for determining abonormalities of shape, Dr. Casanova and coworkers have demonstrated abnormalities of the shape of the temporal lobe in schizophrenia, but the significance of this finding remains unclear. Using the three dimensional technique they have found that the volume of the corpus callosum is not abnormal in this disorder contrary to post mortem data from another laboratory.

A number of postmortem histochemical studies have been performed with some interesting findings. Metenkephalin concentrations are increased in the substantia nigra of patients with schizophrenia. Dopamine reuptake cites, measure with H-3 mazindol, are normal in putamen. In specimens from suicide victims, normal amino acid concentrations were found in a number of brain regions. However, an interesting finding emerged from a study of heat shock proteins using the two dimensional gel elcetrophoresis technique. Heterozygosity was present in 30 % of brains of subjects who died from causes other than suicide, whereas this existed in zero of the suicide cases (n=21). Another post mortem finding of interest was that hippocampal glutamate binding was increased in brains of alcocholics, especially in those individuals who had died froms seizure secondary to alcohol withdrawal. This finding bears on the issue of the role of excitatory amino acid neurotransmitter systems in seizures.

Because of the interest in prefrontal cortical function in schizophrenia and our data about prefrontal dopaminergic activity and prefrontal function, we have been exploring the relevance of prefrontal function to limbic dopamine acitivity. Animal data from other laboratories have suggested that if prefrontal dopaminergic function is reduced, limbic dopamine activity is increased. Dr. Jaskiw has shown that this effect can be reproduced in the rat by deeferenting the prefrontal cortex using the excitatory amino acid neurotoxin- ibotenate. A variation on this theme is evidenced by a study in which Dr. Jaskiw infused the dopamine agonist apomorphine into prefrontal cortex of the rat and observed with the cerebral microdialysis technique reductions of striatal dopamine metabolism. These provacative animal experiments suggest a potential animal model for some of the neurobiological findings in schizophrenia, particularly prefrontal disfunction and dopaminergic overactivity. We expect to continue to pursue this in more claborate animal designs.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders & Stroke

October 1, 1987 - September 30, 1988

The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; and (3) direction of a program of laboratory animal medicine and care (NIMH only). The Branch is comprised of the Section on Instrumentation and Computers and the Section on Laboratory Animal Medicine and Care.

SECTION ON INSTRUMENTATION AND COMPUTERS

The Section on Instrumentation and Computers (ICS) provides technical support for investigators of NIMH and NINCDS IRP's by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing; and (4) managing a central computer facility consisting of a multiuser VAX-11/750, two image processing systems, and a network of Macintosh personal computers and LaserWriter printers.

Additional services provided by the Section include consultation on measurement techniques, signal processing, mathematical and statistical analysis techniques, and equipment and computer purchases. Formal or informal instruction for individual investigators or groups are offered by Section personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

When an investigator requires the services of the Section, he first meets with the Section Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether ICS will take on the project. If a commercial product will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, ICS will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Section Chief or the Assistant to the Chief agrees to accept a project, the investigator submits a standard ICS work request form, signed by his Lab Chief. This form states the nature of the instrument or service requested, and should contain as many details and specifications as the investigator can provide. The project is then assigned to an engineer or computer staff member, who confers with the investigator to formulate a complete set of specifications and a cost estimate for the project. ICS does not charge for services, but upon completion of the project the investigator's laboratory or branch is billed for the cost of the components used. Reimbursement of funds takes place at the beginning of the next fiscal year.

INSTRUMENTATION

The Section has a staff of five engineers, five computer specialists and five technicians to design and produce special-purpose instrumentation. The recent availability of powerful, low-cost personal computers and single-chip microprocessors has broadened the Section's approach to instrumentation development. It is often appropriate for an engineer, a technician, and a computer specialist to work together to combine unique electronic or mechanical hardware, a personal computer or microprocessor, and custom software to produce a flexible, cost-effective solution to complex instrumentation problems. The following are brief descriptions of the Section's major projects, taken from a total of 309 projects undertaken this year.

PATIENT MONITORING/STIMULATION

Ambulatory Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the hardware and software which forms the system.

Monitor. The current version of the PAM has a 1K-byte memory and is in its sixth year of production. Approximately 150 monitors are in use, with the Section providing battery changes and repairs as needed. In response to the need for increased memory capacity and finer time resolution, an improved monitor is under development. The circuit design and breadboard testing of a monitor with a 32K-byte memory has been completed. This design will allow the acquisition of one minute activity values for 22.75 days, while retaining the extremely low battery drain of the current monitor. More advanced printed circuit board techniques will be used to maintain or reduce the monitor's size and to improve reliability. If the monitor's size can be significantly reduced, a smaller case will be developed for it; otherwise the present injection-molded case will continue to be used. Double coating these cases, first with a conductive paint followed by a nonconductive paint, appears to provide adequate rejection of interference from static electricity while protecting the patient from the irritation of the conductive paint.

Computer Support. The Section supports a PAM readout system based on the Macintosh personal computer coupled to a microprocessor-controlled serial interface. A comprehensive PAM program has been written for the Macintosh to handle data readout and disk filing, graphical data editing, construction of continuous data files and raster plots, and formation of tabular data sets for transfer into spreadsheet and statistical applications. During this year the PAM program has been further enhanced and made more reliable. Activity plots can now be saved in MacDraw format, allowing easy editing prior to publication. The program also takes advantage of the larger Macintosh monitors. Ten of the PAM interfaces for the Macintosh have been fabricated for IRP studies; an additional five units have been supplied in support of collaborative efforts outside of the IRP's. Parallel with the development of a new monitor will be the development of a new Macintosh readout program and serial interface to accommodate the PAM's increased memory capacity and to take advantage of its much higher readout speed.

Posture Control System. A system was developed to automate a neurological test for posture control studies in the Clinical Center's Gait Lab. This standard test consists of a quick "push" to the middle of the back of the subject and an observation of the response. The equipment includes a torque motor system used with a mechanical apparatus to produce an adjustable "pull" to a harness attached to the subject. The investigator can set the amount and duration of the "pull" and can introduce a delay to prevent the subject's anticipating the event. A force transducer records the actual force of the "pull" and its time course. A tri-axial accelerometer system is used to monitor torso responses.

Audio Stimulus System. An interface was designed to integrate the use of two portable cassette recorders in presenting an audio stimulus task to a subject undergoing a PET scan. The first tape recorder plays the stimulus tape into the subject's head phones. Prior to starting the stimulus tape, the investigator uses a microphone input to instruct the subject. The subject responds to the stimulus task with a pushbutton which generates a 1 KHz tone that is summed with the stimulus and recorded on the second cassette recorder. This second tape is analyzed off-line to score the responses. The interface included a VU meter to monitor the stimulus levels presented to the subject.

Visual Stimulus Timer. This device provides the selectable timing sequences for the control of three slide projectors used to present a complex visual stimulus. The projectors are all focused on the same screen and present consecutively a stimulus slide, a fixation point, and a response target. The subject responds with a 4-button console to indicate his choice of target. A magnetic stimulator is attached to the subject's head and is activated during the presentation so that its effects on the response time can be studied.

Airway Interruption Device. As part of speech therapy research, the flow of air from a patient's mouth during vocalization is measured as well as the loudness of sound generated. A device was developed to allow periodic interruption of this air flow by a shutter mechanism. The resultant air pressure and any sound produced are measured and recorded. The point of interruption is randomly selected so that the subject cannot anticipate the blockage of air and prematurely stop vocalizing.

ANIMAL MONITORING/STIMULATION

Microprocessor-Based Wheel Rotometer. A 32-channel system for monitoring the running-wheel rotations of rodents has been developed by utilizing an 8-bit microprocessor and a Macintosh personal computer. A cam attached to each wheel axle activates a microswitch to sense the rotations. The microprocessor debounces the 32 switch inputs, determines complete rotations, pulses the corresponding front panel indicators, and transmits to the Macintosh the accumulated rotations for each channel. The Macintosh program determines the accumulated rotation time intervals, stores and formats the data for further analysis and provides real-time histogram displays.

Multispike Interface Device. This bidirectional interface device was designed to aid the development of a multispike analyzer for animal vision studies based on an AT&T DSP32 Digital Signal Processor and an IBM-AT PC. The interface provides amplification, a 6-pole anti-aliasing filter, a sample/hold amplifier, and a 12-bit, 10 μsec. A/D converter to process the incoming spike train at either 20 or 40 KHz. The sampled values are then transferred to the synchronous high-speed (2MHz) serial port of the DSP32 for digital processing. When a spike is detected, two 12-bit values are shifted from the DSP32 high-speed serial port into the interface for conversion to three 8-bit bytes which are then transferred to the parallel interface within the AT. For control and down loading of programs, the AT and the DSP32 communicate through RS-232C serial ports.

Biphasic Isolated Stimulation Unit. A stimulation unit has been designed to accept unipolar voltage pulses from standard lab generators and to generate biphasic isolated constant-current pulses with 100-volt compliance. The amplitude and duration of the original pulse are preserved and used to generate a second pulse with opposite polarity whose amplitude and duration can be set within a 25–250% range of the original pulse. The high-voltage output stage is isolated by optically coupled amplifiers and DC-DC power inverters. Peak output currents can be set from 1-1500 μA; actual peak values are sampled and displayed on digital meters. Line voltage operation is selectable at 120 or 240 volts.

CONTROLLERS/TIMERS

Motor-Controlled Solution Changer. A device has been developed to allow rapid changes in the ionic composition of the extracellular fluid surrounding isolated cells. A powerful stepper motor drives a hydraulic micromanipulator which carries a linear array of micropipettes. The solution in each barrel of the array is driven at the same flow rate by a multichannel pump and is gated on and off by an electronic valve controller that was developed last year. The motor controller presently allows rapid switching between a selected set of two barrels (~100 msec. with 400 μm dia. barrels). Additions under development will provide for a complex pattern of movements involving a selected set of three barrels and will include the corresponding timing patterns for the valve controller.

Temperature-Controlled Perfusion Chamber. A collaborative effort with the LDN, NICHD has resulted in the development of an environmental chamber that permits perfusion and temperature control of the extracellular medium during electrophysiological studies of cultured or acutely dissociated neurons in 35 mm petri dishes. The chamber employs a complex Plexiglas holder and base unit and a two-piece peltier-driven brass heat exchanger. The dish temperature may be regulated above or below ambient by thermistor feedback to the peltier power supply. Twelve of these improved perfusion chambers have been fabricated this year for use in four intramural laboratories.

Pneumatic Occlusion Device. For studies involving temporary brain ischemia, a device was developed to allow precise movement control over a set of small weights used for carotid artery occlusion. An electrically-activated valve allows electronic timing control of a two-position pneumatic cylinder which moves a platform supporting the weights. Adjustable flow control valves provide smooth platform movements and a novel contact switch arrangement gives precise timing of the occlusion.

Battery Discharge/Charge System. NiCad battery packs are used in numerous ambulatory temperature monitors during clinical studies. Obtaining reliable battery performance with the manufacturer's charging system has been difficult under clinical conditions. A four-channel unit has been developed to correct this problem by ensuring proper battery discharge/charge cycles. After a monitoring session, the battery pack is first fully discharged at a high rate until the correct terminal voltage is reached. The batteries are immediately charged for 14 hours and then maintained with a trickle-charge until needed again. Two additional four-channel units are being fabricated to support the large number of clinical applications.

Dual-Display Testing Timer. A compact dual 4-digit LED display timer has been developed for use in a monkey memory testing apparatus. The first display shows the investigator the total elapsed time of the testing session. It can be switched to a HOLD mode or reset as needed. The second display is controlled by a microswitch attached to a sliding door. When the door is lowered, the timer is switched on so that the investigator can accurately control the time that the animal has to remember the objects that it has just seen when the door was raised. Six of these units have been fabricated for this application and the timer printed circuit board has also been useful in other projects.

COMPUTER SUPPORT

In addition to the development of special instrumentation systems, ICS provides support for individual laboratory computer systems and maintains central computer facilities for high-capacity data storage, complex off-line data analysis, image processing, scientific word processing, and high-quality printing and plotting. These support services are detailed under the following categories.

LABORATORY COMPUTERS

Small minicomputers, and more recently, personal computers, are widely used in the IRP laboratories for real-time data acquisition and control. ICS provides consultation on the specification and selection of these systems and helps the scientist in the procurement, installation and maintenance of the equipment. Training in operating systems, programming languages and maintenance issues is available for scientists or laboratory support personnel. Manpower limitations make it difficult for ICS to provide complete programming for specific individual applications. Section personnel are always available for consultation and will aid the investigator in writing the difficult time and data dependent sections of real-time programs. Section programming efforts in support of laboratory applications are concentrated on developing and maintaining a library of routines which are specifically designed to be incorporated into investigators' programs. ICS personnel also evaluate commercial software or applications programs from other research facilities to determine their utility for IRP laboratory systems.

ICS has selected the Apple Macintosh family of computer systems as our standard for support of scientific applications. The Section has developed considerable experience using the Macintosh Plus to provide innovative solutions for low-speed laboratory data acquisition projects. For the acquisition of real-time data and control of laboratory devices at high speeds, the Section has begun development of utility routines and applications software for the Macintosh II. The goal is to develop a laboratory data acquisition and control system which provides equivalent features to those on older systems now in use, and to provide extended capabilities by utilizing the advanced graphical features of the Macintosh II. This system, called MacNeuros, is being modelled on a PDP-11 system that has evolved through the efforts of Section staff and numerous individuals in the laboratories of NIMH, NINCDS, NICHD, and NIAAA. System specification suggestions have been solicited from these groups. As before, modular routines are being developed to handle high-speed data input and output, to generate on-line graphical feedback, and to provide off-line data analysis with publication-quality tables and figures. General purpose analog/digital input/output boards for the Macintosh II have been evaluated from three manufacturers for their suitability for MacNeuros. More advanced boards will be evaluated as they become available. Efforts are being made to keep the I/O routines flexible enough to accommodate a variety of boards so that the most appropriate one can be selected for a particular laboratory application.

VAX FACILITY

ICS manages a DEC VAX-11/750 computer system that is available to all IRP investigators. VAX/VMS is the primary operating system, though a System V UNIX shell with Berkeley extensions is also available. Over 50 users in Bldg. 36 and Bldg. 10 have hard-wired cable connections or leased lines for high-speed communication with the VAX. Users can also gain access on a dial-up basis at 1200 or 2400 baud. Neurophysiologists are using the system for modelling and graphing neurophysiological data. Molecular biologists and biochemists are analyzing and editing DNA and protein sequences and preparing them for publication. These scientists are also searching large biological sequence databases, comparing sequences for biological similarity, and plotting the predicted physical structure of nucleic acid and protein sequences.

Currently, the most popular package on the VAX is the sequence analysis software from the University of Wisconsin Genetics Computing Group. This package includes about 100 programs, a 579 page illustrated manual, and complete on-line help. The Section also provides the complete GenBank nucleic acid database and the NBRF protein database with quarterly updates.

Another popular program on the VAX is DataPlot, an interactive program for curve fitting and graphics. Similar to MLAB, DataPlot is a tool for experimenting with mathematical models, as well as summarizing and analyzing data. Publication-quality graphs produced by DataPlot can be plotted on a Talaris laser printer using utilities created with the Section's PlotLib graphics library.

In order to meet the increasing demand for VAX processing power, NIMH and NINCDS have jointly funded the purchase of a VAX 3600 system that will be much faster than the six year old VAX-11/750, and will provide an additional 600 MB of disk storage and a new 300 MB tape drive for file backup. All functions currently performed on the 750 will continue on the new system. Additionally, a software package called AlisaTalk is being purchased for the 3600 that will provide central network file service, terminal access, and print spooling for IRP personal computers. Equipment has been ordered which will allow PDP-11 laboratory computers and personal computers to access the 3600 using high-speed Ethernet cabling. Personal computers on the ICS AppleTalk network will have access to the 3600 via an AppleTalk-to-Ethernet gateway. AlisaTalk and Ethernet will allow experimental data to be rapidly transferred to the 3600 for analysis, plotting, and archival storage.

IMAGE PROCESSING FACILITY

The Section maintains a central facility in Bldg. 36 for image processing consisting of a PDP–11 computer, a rotating-drum scanner, and a image array processor. This system has been heavily used for numerous applications, including evaluation and quantification of CAT or ECAT images, densitometric analysis of autoradiographs, and analysis of two-dimensional electrophoresis gels. Unfortunately, this system has now become outdated, unreliable, and very expensive to maintain. The Section is in the process of developing a Macintosh II and video camera based system which will have most of the capabilities of the PDP-11 system, but will be smaller, more reliable, significantly less expensive, and much easier to use. Central to this system is a Macintosh II program called Image which is being developed by Section personnel for acquiring, enhancing, analyzing, editing, animating, and pseudocoloring images. Image already has almost all of the general image processing capabilities of the program of the same name that runs on the PDP-11 system. It will eventually have the ability to perform the quantitative densitometry required for cerebral glucose utilization and receptor binding studies. Image is currently being used by two IRP laboratories for editing and enhancing receptor binding autoradiographs and for molecular modelling. When the development of the Macintosh II system is completed, investigators with extensive image processing requirements will easily be able to duplicate it in their own laboratories.

PERSONAL COMPUTER FACILITY

Included in the Section's central facility in Bldg. 36 are three Macintosh Plus computers, a modem, a LaserWriter Plus printer, and a variety of software that IRP scientists can use for statistical analysis, for communicating with DCRT's mainframes and MEDLINE, and for word processing, including creation of posters, slides, and publication-quality charts and graphs. All three Macintoshes are also connected to the Section's VAX and can be used to emulate VT-100 or Tektronix 4014 terminals.

COMPUTER NETWORKS

Appletalk Network. ICS has continued to expand the network linking Macintosh and IBM clone computers via the Appletalk protocols. The network was started within ICS in Bldg. 36, and the network server is located there. Branches of the network have now been established in Bldg. 10

and Bldg. 9 over dedicated phone lines used as data lines. The branches communicate at Appletalk speed, 230K bits/sec., over the unshielded twisted-pair lines. The network presently encompasses the Section on Laboratory Animal Medicine and Care in Bldg. 9; the Office of the IRP Director, the Information officer, and the Section on Clinical Neuroendocrinology in Bldg. 10; the Research Services Branch, the Unit on Functional Neuroanatomy, and the central computer facility in Bldg. 36. In addition, 2400 baud remote telephone links will be established with the Office of Scientific Information, NIMH, and the Division of Personnel Management, ADAMHA, in the Parklawn Building. Other labs and branches with Macintosh computers and requirements to communicate with other labs or the IRP OD will be added to the network in the next year. The cost is minimal: about $700 per lab for a network bridge, plus $50 per machine for connectors.

3-COM Token-Ring Network. The Office of the NIH Director, Bldg. 1, with the support of the DCRT Personal Workstation Office, is establishing a network based on the token-ring technology. It is anticipated that all administrative offices at NIH will ultimately communicate with this network via the NIH fiber-optic broadband network currently planned by DCRT to link all buildings on campus. In anticipation, ICS is prototyping a token-ring network, with the following goals: (1) establish the techniques required to connect to the NIH and OD networks; (2) provide access to the NIMH/ADAMHA Novell Ethernet network in the Parklawn Building; (3) develop in-house expertise in token-ring technology, with the possible eventual goal of designing a network to link all the administrative and lab/branch offices in the IRP; (4) develop expertise in multiuser network based software for financial management, personnel applications, and database management; and (5) determine the feasibility of upgrading outmoded PC's for network use.

COLLABORATIVE SUPPORT

Section computer specialists provide collaborative support for selected research projects within the intramural laboratories. These efforts and the resulting software developments are described below.

Morphological Classification of Neural Cells by Fractal Geometry. A collaborative effort is in progress with the LNP and the LNC, NINCDS and the LDN, NICHD to use fractal geometry as a mathematical basis for the quantitative classification of neural cells grown in culture. An edge detection technique has been developed to isolate and outline digitized cell images which retains as many of the branches (dendrites and axons) as possible. Three computational techniques have been found that accurately calculate the fractal index of known fractal images and are consistent when used to measure tree structures of similar construction and within types of neural cells. An alternate method based on Fourier transform theory is also being investigated. Additionally, other measures that reflect the contribution of the cell body as opposed to the dendritic processes are being evaluated.

Flow Cytometry Studies. A comprehensive collaborative effort is in progress with the LNP, NINCDS which involves the use of voltage-sensitive dyes and flow cytometry techniques to study the electrical and pharmacological properties of embryonic rat CNS neurons. Section effort is focused on experimental design, statistical and graphical analysis of data, and the development of custom software.

Helix. A Macintosh program called Helix has been developed which calculates the most hydrophobic portion of a given amino acid sequence, configured as a helix. The user specifies the helix type (a, p, or 3_{10}), the physical parameters to be used in the calculation (up to 10), the amino acid sequence file and the starting and ending position of the amino acid sequence in the file. The program places the residues in the appropriate positions on the helical wheel, calculates the mean helical hydrophobic moment, and determines which half of the helix is most hydrophobic and thus most likely to be buried in the membrane. Helix produces both a MacDraw and a text document.

Protein Sequence Layout. A Macintosh program was developed to represent protein sequences as a chain of balls identified by the appropriate initial. These chains may be easily moved about on the screen to show the spatial configuration of a sequence. Individual proteins may be highlighted by shading. The chain may be superimposed on a figure, such as a cell membrane, and printed.

Three Dimensional Plots. Data sets generated from commercial statistical programs may be entered into this Macintosh program and displayed on three axes in three dimensions. The axes may be interactively rotated to find the best aspect ratio.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care (SLAMC) provides a comprehensive animal care and use program for the NIMH Intramural Research Program. The average daily animal inventory is 2,400 animals, including mice, rats, hamsters, cats, guinea pigs, frogs and nonhuman primates. The Section is responsible for 22 rooms, comprising approximately 10,000 sq. ft. The staff includes one veterinarian, one facilities manager, one biologist, eleven full-time animal caretakers, three part-time animal caretakers, and one secretary. Approximately 250 IRP investigators are involved in animal research, and depend on the Section for supervision and advice on animal care and use issues.

SLAMC is involved in numerous program activities required by ADAMHA, NIH regulations, and PHS Policy. SLAMC will:

Provide adequate veterinary care. Twenty four hour clinical care is provided for all IRP animals. This includes provision of routine and emergency medical and surgical care. An animal health surveillance program is also in effect . For rodents this involves semiannual serology and pathology. For primates this involves quarterly TB tests and physical examinations. Hematology, clinical chemistries, dental care and serum banking are done at least annually on primates.

Provide attending veterinary services for the William A. White facility at St. Elizabeths Hospital. St. Elizabeths is negotiating to obtain coverage from a veterinarian who works in close proximity to the facility.

Maintain clinical records. A system of maintaining clinical records for all primates has been developed and is computerized.

Maintain an employee health surveillance system. The Section requires all persons who come in extensive contact with animals to enroll in the Animal Exposure Surveillance Program.

Maintain Animal Study Protocol forms. The Section monitors the documentation and approval process for animal usage and maintains records of all ongoing animal studies.

Participate on the Animal Care and Use Committee. The Section is represented at all Committee meetings, provides full documentation of the deliberations, and provides the administrative support for the committee.

Perform animal facility site visits. Formal site visits are conducted semiannually, and appropriate documentation of findings is provided.

Adhere to the NIH "Guide for the Care and Use of Laboratory Animals". The Section is responsible for monitoring IRP compliance with the NIH regulations in the Guide.

Strive for animal facility accreditation. A plan for AAALAC accreditation of the facilities at NIH, St. Elizabeths, and Poolesville is being developed.

Manage the day-to-day operations of the animal facilities.

Oversee surgical facilities. The Section assists investigators with anesthesia and surgical techniques.

Provide space for storage of animal care equipment.

Provide animal room sanitation. Adequate sanitation of all animal facilities is provided. Cage washing is provided in Bldg. 9.

Provide for training of personnel. The Section provides information to Lab Chiefs concerning the availability of approved courses for investigator training required by PHS policy. The Section makes provisions for animal care personnel to receive AALAS training leading to certification as an animal technician or technologist.

Prepare the Annual Report of Research Facility as required by the USDA.

Prepare the Annual Report of Research Facility as required by ADAMHA.

SLAMC will also:

Prepare AAALAC-accreditable plans (including blueprints) for Bldg. 14D', and work with other veterinarians to develop plans for Bldgs. 36 and 10A.

Maintain experimental testing rooms. The Section provides and maintains two primate experimental testing rooms.

Provide animal and space inventory system. The Section monitors usage of animal space by maintaining a comprehensive and current inventory of animal holdings.

Manage an animal ordering system. The Section operates a centralized system for ordering animals.

Assist investigators with locating and procuring nonhuman primates for research.

Manage a Rhesus monkey nursery facility, attended 15 hours/day, every day.

The Section maintains membership and active involvement in the following committees: NIH Animal Care and Use Committee; NIMH Animal Care and Use Committee; Animal Programs Advisory Committee; Task Force Committees for Bldgs. 10A, 14D', 36, 49, and 110A at Poolesville; SAIDS Task Force Committee, and the Animal Issues Committee. The Section also maintains involvement in the American Association For Laboratory Animal Science.